Evaluation of Enzymatic Breakers for the Reduction of Environmental and Health Hazards Associated with Hydraulic Fracturing Fluids

THESIS

Presented in Partial Fulfillment of the Requirements for Graduation with Honors Research Distinction in Chemical Engineering at The Ohio State University

by

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2016

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Abstract

Hydraulic fracturing ("fracking") is the process in which highly-pressurized fluids are injected underground to obtain energy resources that could not otherwise be accessed. Under high pressures, fissures are created in rock formations, which free trapped energy resources. The fluids used are composed mostly of water with chemical additives, which each add a specific functionality. A polymer increases fluid viscosity to initiate the fracture. A crosslinker may be added to further increase viscosity. Breakers decrease fluid viscosity for flowback. Despite its ability to retrieve critical energy resources, a primary concern with fracturing is its impact to human health and the environment. Many fluid components are toxic and there is fear that the fluid may contaminate groundwater. The goal of the research was to identify alternative components for fracturing fluids. Specifically, breakers were examined. Current breakers are strong oxidizers which degrade the polymer by a free-radical mechanism at high temperatures. Enzymatic breakers, however, are benign to environmental and human health. Two cellulasic enzymes, β-mannanase and α-galactosidase, were proposed as alternative breakers and their performance was compared to that of ammonium persulfate, the industry-standard breaker. Polymer (guar) and crosslinked polymer fluids were broken at well conditions ($50^{o}C$) and industry-standard concentrations over time (18 hr.). Rheological testing of these fluids included frequency sweeps and steady shear rate sweeps to determine viscosity change over time and breaker kinetics. Filter cake testing was performed to study polymer degradation over time, as the breaker liberated low molecular weight oligomers. Statistical analyses were used to analyze these results. Enzymes show some promise in competing with ammonium persulfate as a breaker, although further testing is necessary. More environmentally-friendly components for fracking fluids may allow fracking, important to the U.S. energy portfolio, to continue with lessened risk and concern.

Acknowledgements

I would like to thank my family, friends, and loved ones for their continual and untiring support and encouragement. Truly, this was not possible without you.

To my research advisor, Dr. Kurt Koelling, I would extend thanks for providing me with the opportunity to pursue undergraduate research. I have learned much and credit his mentorship with helping me to become an independent, disciplined, and inquisitive scientist and student and guiding me toward a career in research.

To the other instructors and mentors that I have had during my time at Ohio State, I would offer thanks. You not only taught me the fundamentals of the discipline, but through constant challenge, showed me how to become a successful engineer.

I thank the College of Engineering and the University Honors Program, as well. Their support, financial and otherwise, of my education is greatly appreciated.

I cannot forget about my many lab peers that have always assisted me in times of need or doubt or simply offered friendship: Benjamin Crum, Joseph Gauthier, Nicholas Ohanian, Rocky Wang, Andrew Newmyer, Steve Shi, and Jack Jones.

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Field of Study

Major Field: Chemical & Biomolecular Engineering

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Introduction

Background

Hydraulic fracturing ("fracking") is the process in which highly-pressurized fluids are injected deep beneath the ground to obtain energy resources (e.g., oil, natural gas) that could not otherwise be accessed by conventional methods. Under these high pressures, fissures are created in the rock formations, which then propagate and open pockets where the resources are trapped. Once freed, the resources migrate to the surface, where they can be captured and stored for future use [1]. In the United States, fracking typically aids in the retrieval of natural gas from shale. Shale plays range from the Great Lakes, to the South, and the Great Plains [2]. Figure 1, below, shows a map of the various shale plays of the United States.

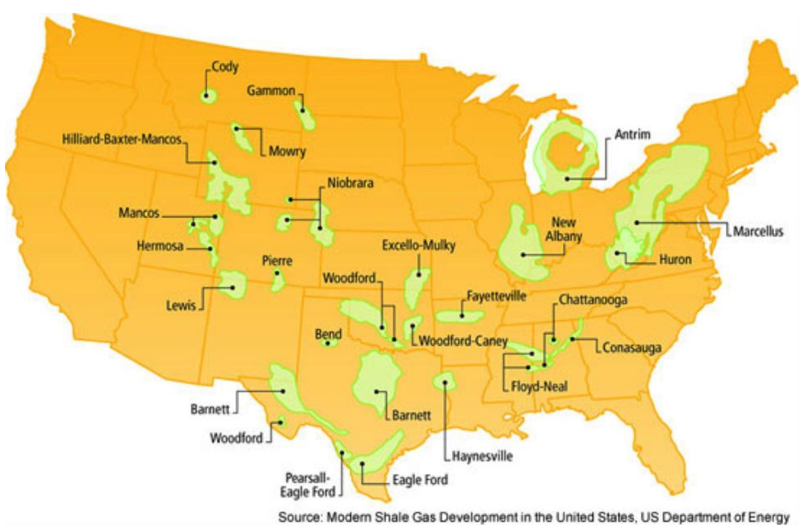

Figure 1: Map of shale plays in the United States **[3]**.

Fracking is credited with being at least partially responsible for the recent energy boom in the United States. Further, with an estimated 200 trillion cubic feet of recoverable, domestic natural gas, fracking has been, and is predicted to continue to be, an important and reliable part of the United States' energy portfolio [2] [4].

Most fracturing wells begin by drilling a cement-lined hole to the desired depth, where the resource in question is located (6,000-10,000 ft. downward), with encasings inserted to protect the water supply (1,000-4,000 ft. downward) [3] [2]. Temperatures here are 25-150^{o}C [5]. A key characteristic of a fracking well is a horizontally-drilled section, which essentially increases the surface area the well is exposed to the shale and makes it more accessible to fracturing [3]. Once the well is completed, large volumes of the fracturing fluid are pumped down to cause the fracture. A single fracking well could require 2-4 million gallons of water for operation [3]. Figure 2, below, shows a diagram of such a fracking well in use.

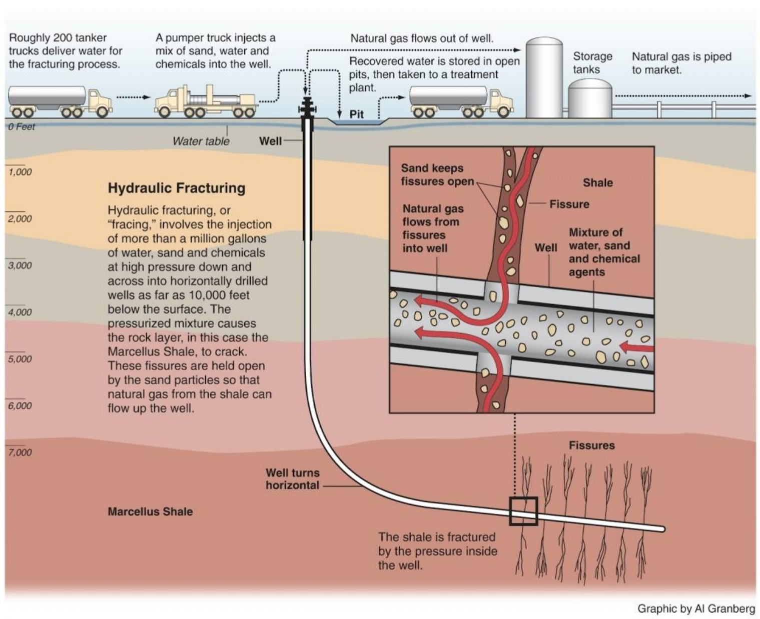

Figure 2: A hydraulic fracturing well **[6]**.

Fracturing fluids consist mostly of water (~99%) and many chemical additives present in trace amounts, which each add a specific function [1]. Polymers (most commonly, guar) increase the viscosity to perform the fracturing and transport proppant; cross-linking agents further increase fluid viscosity. Biocides prevent bacterial fouling of the well, while scale inhibitors and stabilizers maintain well conditions. Two important components are the proppant, which holds open the fissures for the resource to escape once pressure is released, and the breaker, which, following the fracturing, degrades the fluid and decreases viscosity for extraction [2]. One concern with fracking is that this fluid, which contains many potentially harmful chemical species, may contaminate the groundwater and adversely impact human health and the environment. This may be caused by faulty encasings, bleed of fractures into the water supply, or improper fluid disposal or storage [2]. Additional concern is with the fluid that remains in the well, as 30-50% is left as residue. High residue both limits the possible product yield by decreasing proppant conductivity and poses environmental concerns [4].

To facilitate flow of the energy resource from the well, the fracking fluid must effectively transport the proppant particles to the fissures (especially at these long distances). Because the proppant is more dense than water, the viscosity of the fluid must be higher than that of water to prevent undesirable settling. Hence, a polymer is added as a viscosifier [7]. Hydroxypropyl guar, a plant-derived, hemicellulosic polymer, is the standard choice. Historically, guar has been used in the food, textile, and paper industries and has seen expanded use in the petroleum industry in recent years [8]. This is because, compared to other water-soluble polymers, guar is thermostable, has good cleanup properties, and is robust at a range of shear rates, salt concentrations, and pH. Additionally, guar has a high molecular weight (1-2 million on average) and increases viscosity at relatively low concentrations [8] [7]. Figure 3, below, shows the chemical structure of guar.

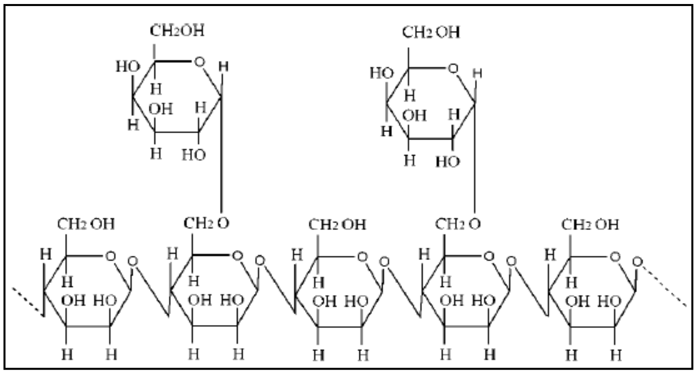

Figure 3: Structure of hydroxypropyl guar **[9]**.

Guar is composed of a linear backbone of β-1,4-linked mannose units with random α-1,6-linked galactose unit side chains. The ratio of mannose to galactose units is 1.6:1 to 1.8:1 [9]. To further increase viscosity and the effectiveness of the fracking fluid, the guar may be cross-linked, as well. Ionic salts, such as boron and zirconium salts, may be used to link two (or more) polymer chains together to create a gelled network, which increases the effective molecular weight and, thereby, the viscosity of the fluid. The effectiveness of the cross-link can depend upon metal ion, temperature, and pH and linkages can occur between guar's main and side chains [8]. The mechanism in which guar is cross-linked is shown below, in Figure 4. The typical choice of cross-linking agent is sodium tetraborate [8].

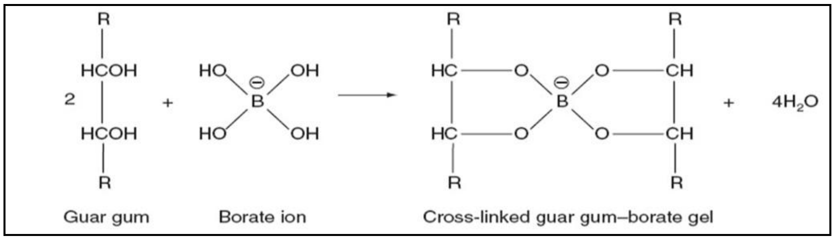

Figure 4: Mechanism of guar cross-linking with borate ion **[10]**.

Motivation

The importance of fracking in the United States cannot be downplayed. Fracking offers an abundance of recoverable energy resources, enough to secure the nation's energy independence and provide its needs for the next 90-115 years; this could allow enough time to develop alternative, more renewable energy sources [3]. In fact, today, 86% of the United States' natural gas production comes from enhanced recovery methods, such as fracking [3]. The natural gas retrieved by fracking is the most environmentally-friendly fossil fuel, as well. Methane, when combusted, produces less CO_2, SO_x, and NO_x than other fossil fuel and coal [11].

However, as mentioned, fracking is not without its issues. There is a recognized concern that many of the chemical components in fracking fluids are toxic in only moderate concentrations and there is a possibility the fluids may contaminate the groundwater [12]. Even in the case that the wells do not leak, fracking uses incredible volumes of water that are difficult to re-use or purify, as any toxic components remain in the water. Once removed from the ground, the water now contains naturally-occurring radioactive materials [13]. Storage, disposal, and treatment of these fluids are ongoing concerns. Loss events have the potential to cause long-term damage to the surrounding communities and ecosystems [13].

Breakers are some of the most hazardous components of the hydraulic fracturing fluids currently used [12]. The breakers used in industry are predominantly strong oxidizers, which promote polymer degradation via free-radical redox reactions at elevated temperatures (above 50^{o}C) [14]. Oxidative breakers, with ammonium persulfate the most prevalent, are recognized as toxic to humans and the environment and are highly combustible [12]. Figure 5, below, shows the mechanism by which guar is broken by oxidation using ammonium persulfate.

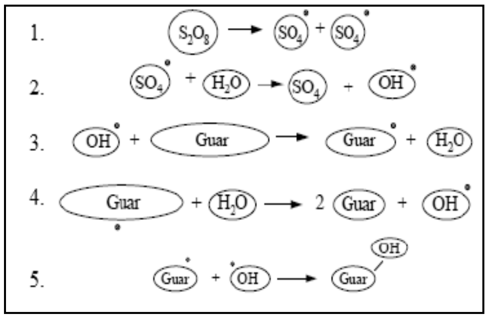

Figure 5: Mechanism by which guar is broken by ammonium persulfate **[14]**.

Significance & Purpose

An alternative to conventional breakers, however, are enzymatic breakers. Enzymes are generally regarded as safe to biological organisms, are catalytic (allowing them to be used in low concentrations), are of large molecular weights (allowing them to remain with the guar fluid, rather than filter into the rock formation), and are less susceptible to the effects of contaminants in the fluid [9]. The drawback is that enzymes are far more sensitive to temperature and pH than oxidative breakers. This limits their robustness and wide use in industry [15].

Being a plant-derived, natural, hemicellulosic polymer, guar is capable of being degraded by such enzymatic breakers. The mechanism by which this occurs is shown below, in Figure 6. The principle is much the same as an oxidative breaker: the polymer chains (either side chain or main chain) are degraded by breaking or cleaving units, which decreases the molecular weight, and thus, the viscosity [9].

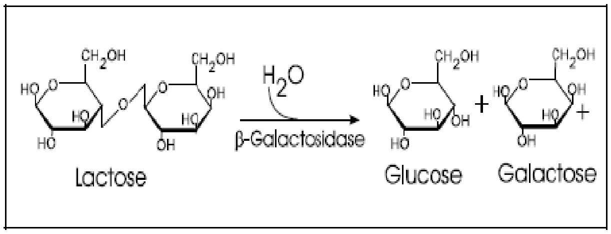

Figure 6: Mechanism by which guar is broken by an enzymatic breaker **[16]**.

The three bonds in guar (see Figure 3) susceptible to enzymatic degradation are: the endo- and exo-β-1,4 linkages between mannose units (bonds of the main chain) and the α-1,6 linkage between galactose and mannose units (bonds between the main chain and side chain residues). The enzymes that are capable of cleaving these bonds are β-mannanase and α-galactosidase, respectively. These enzymes were identified and proposed as alternatives to oxidative breakers [9].

The chemical structures of the enzymatic breakers, along with the conventional oxidative breaker, ammonium persulfate, are shown in Figures 7, 8, and 9, below.

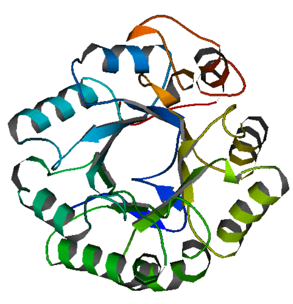

Figure 7: Structure of β-mannanase **[17]**.

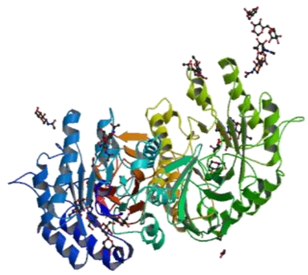

Figure 8: Structure of α-galactosidase **[18]**.

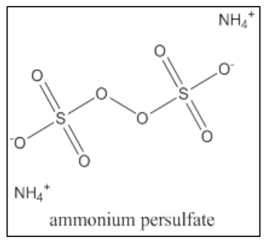

Figure 9: Structure of ammonium persulfate.

The two proposed enzymes are typically used in breaking down complex biomass, for example, to enhance nutritional value in livestock feed [19] [20]. They are biologically benign. In fact, α-galactosidase is reported to be a digestive aid [18]. Clearly, these enzymes are more desirable breakers than ammonium persulfate.

The goal of this research was to evaluate enzymatic breakers that may be used as environmentally-friendly alternatives to oxidative breakers in hydraulic fracturing fluids. Replacing potentially-harmful components of fracking fluids, like oxidative breakers, will contribute toward the elimination of health hazards posed by fracking and lessen concerns with this key enhanced energy recovery method.

Literature Review

The concept of using enzymatic breakers in hydraulic fracturing fluids is not entirely novel. Nasr-El-Din et al., for example, attempted to use enzymatic breakers to minimize residue, which can decrease well efficiency. It was found that enzymes take longer than oxidative breakers to act and that break time is a function of breaker type, concentration, and polymer loading [21]. Sarwar et al. conducted extensive testing with several oxidative breakers, as well as an enzymatic breaker, galactomannanase (likely β-mannanase). It was found that, from testing over a wide range of temperatures and concentrations, galactomannanase was best at breaking at ambient conditions and eliminating the most residue. Additionally, decreasing viscosity does not necessarily mean molecular weight has been decreased, as fluids can contain large polymer chain fragments [5]. Zhang et al. engineered and tested a genetically-modified β-mannanase enzyme for use in fracking; essentially, the enzymes were engineered to be more thermo- and pH-stable. The enzyme was determined to be able to compete with ammonium persulfate in terms of well conductivity, residue reduction, and rheology [22]. McCutchen et al. conducted similar studies, designing and evaluating genetically-engineered β-mannanase and α-galactosidase enzymes. It was found that the enzymes, and a synergistic mix of the enzymes, can degrade guar [23]. Cheng and Prud'homme conducted kinetics studies on β-mannanase on guar substrates and looked into the enzymatic breaking mechanism, especially in the presence of concentrated and highly-substituted polymers, and how it is affected by temperature and pH [9]. As the petroleum industry is highly-lucrative, however, it is difficult to get concrete results from the literature and much is kept proprietary. For instance, Gunawan et al. report an "environmentally-responsible, catalytic breaker," that appears to have the potential to completely replace oxidative breakers, but do not disclose its chemical identity or nature (enzyme, or otherwise) [24]. As well, for this research, attempts were made to procure the enzyme developed by Zhang et al. at Verenium Corporation (subsidiary of BASF) for evaluation. Attempts were met with failure.

Experimental Methodology

Overview

Experimental methods were designed keeping industrial fracking operations in mind; systems used were meant to simulate how fracturing wells exist in practice.

The testing temperature was 50^{o}C, the approximate average well temperature [5].

Breaker concentrations were determined from literature and industrial sources. FracFocus is a chemical disclosure registry for hydraulic fracturing. Either voluntarily or by mandate, companies report information about their wells, including their fluids' compositions. Ammonium persulfate was found to range in concentration between 0.01 and 0.1% by weight. Guar was found to have a standard concentration of 0.5% by weight. Crosslinker was found to range in concentration between 0.08 and 0.25% by weight; 0.25% was chosen so that a large disparity between guar and crosslinked guar could be realized [25]. Sodium tetraborate was the chosen crosslinking agent, as it is relatively standard in industrial practice. Enzyme concentrations were chosen from typical literature values, which range from 0.01-0.1 U/mL [9]. In enzyme chemistry, a unit, U, is defined as the amount of enzyme that catalyzes the conversion of 1 μmol of substrate per minute. It was desired that a synergistic mixture of both enzymes be tested, as well, as the two enzymes act on different parts of the guar structure. The mixture was chosen to be at a concentration of 0.1 U/mL. See Table 1, below, for the chemical concentrations of the simulated fracking fluids used for experiments.

Table 1: Concentrations of chemicals used in model fracking fluids.

Component	**Concentration**	
Ammonium Persulfate	0.01% wt.	0.1% wt.
Mannanase	0.01 U/mL	0.1 U/mL
Galactosidase	0.01 U/mL	0.1 U/mL
Guar	0.5% wt.	
Sodium Tetraborate	0.25% wt.	

Time-scales were determined from preliminary experimentation. It was found that, generally, the time-to-break is no longer than one hour. Although, to record any further breaking, especially from the enzymes, which had not been previously studied, an additional measurement was to be taken at a longer time (18 hours).

Experimentation included comprehensive rheological, degradation, economic, and hazard analyses. Performance relative to each of these parameters are important characteristics of a breaker. Breakers must be able to effectively decrease fluid viscosity, degrade the polymer to leave as little residue as possible, be cost-effective, if to be used in industry, and, ideally, pose few health risks or hazards. Statistical methods were applied, where appropriate, to analyze obtained results.

Thanks is extended to Mianyang Habio Bioengineering Company for providing the two enzymes used in this study and Solvay for providing the guar polymer (Tiguar 418). Additional chemicals sourced were: sodium tetraborate (Thermo Fisher Scientific) and ammonium persulfate (Santa Cruz Biotechnology, Inc.).

It is worth noting that, while enzymes are typically sensitive to pH and temperature and those procured for this study were not genetically-engineered for thermo- or pH-stability, they are reported to have little reduced activity at the temperature and pH of testing here. See Appendix A, Figures A1-A4 for details.

In all studies, fracking fluids were prepared by first mixing the guar (600 RPM) in distilled water for 1 hour, so that the polymer was completely hydrated. If the fluid was to be crosslinked, sodium tetraborate was then added and the solution was mixed for 23 hr. Both fluids were allowed to sit for 24 hr. prior to any testing. Breaker solutions were prepared by mixing each chemical in distilled water.

Rheological Studies

Model fracking fluids (guar or crosslinked guar) were treated with a specified breaker (β-mannanase, α-galactosidase, a mix of the two, or ammonium persulfate, at varying concentrations) and were heated for a prescribed time (0, 15, 30, 45, 60 min. or 18 hr.) in a water bath at 50^{o}C. Doses of aqueous breaker solution (0.5 mL) were added to small volumes of fracking fluid (7 mL for ammonium persulfate tests, 10 mL for enzyme tests) and mixed in sealed vials. For control fluids, 0.5 mL of water was added to negate any effects of dilution.

The fluids tested in this work were non-Newtonian and viscoelastic. This is due to the polymer structure within the fluid. Rheological testing was chosen to characterize these complex fluids and determine how their properties (i.e., viscosity) change over time with the addition of a breaker. The rheological tests performed were oscillatory frequency sweep and steady shear rate sweep tests. These were done using an ARES G-2 Rheometer, pictured below, in Figure 10.

Figure 10: The ARES G-2 Rheometer used for testing.

Oscillatory frequency sweep testing involves shearing the fluid back and forth (imposing a strain wave), in an oscillatory fashion, and measuring the corresponding stress response. Two parameters, G' and G" will be found, which are called the storage and loss moduli, respectively. These are indicators of how "solid-like" or "liquid-like" the materials behave. Complex viscosity can then be calculated from these moduli. [26]. The strain used for these experiments was 50%, which was determined from previous work [27]. Previous work used strain sweeps to determine the ideal strain to run these tests for good results. For these studies, the frequency sweep was from 0.1 to 100 radians/second. This is a practical testing range, as much greater than 100 radians/second would likely not be encountered by these fluids in industry and 0.1 radians/second seemed to be the operational limit of the rheometer; any lower and the results were "noisy."

Frequency sweep testing was first, then, a steady shear rate sweep test was performed. The order of the tests was important, as the high shear imposed on the fluid in the steady shear rate sweep test can deform and alter the fluid's structure. These tests shear the fluid in one direction and the fluid's stress response (torque) is measured. The shear viscosity as a function of shear rate, at a constant strain rate, can be calculated. These tests were performed from 0.1 to 100 s^{-1}, for similar reasons as the first tests. Both of these tests are similar in goal, determining viscosity, but both were performed to gain the most knowledge of fluid properties.

It is important to note that, in these rheological tests, a cone and plate geometry (50 mm diameter, 0.02 radian angle) was used. A diagram of this geometry is shown in Figure 11, below. Different rheometer geometries (e.g., parallel plates, cone and plate, Couette) each calculate the fluid's properties in different ways and each have their respective advantages and disadvantages. Cone and plate geometry offers a uniform shear field and highly-accurate normal force measurements; however, it is highly-sensitive to the gap of the geometry (the space between the plates) [28]. In practice, fluid is placed between the cone and the bottom plate and rheological settings are set in the computer control program.

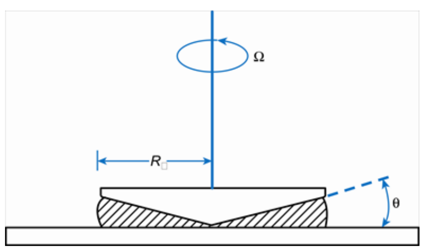

Figure 11: Cone and plate geometry **[29]**.

All testing took place at 25°C (room temperature) and atmospheric pressure. Elevated temperatures are required for breaking, but previous attempts to heat the fluid while being broken and measuring viscosity over time was met with issues from evaporation. Evaporation greatly interferes with viscosity measurements. It proved a better alternative to heat the fluid in closed vials for prescribed amounts of time, then stop the breaking (approximately) by removing them from the heat. However, this mandated that the break time was set in stepped time intervals, rather than being continuous. Even if the break was not occurring at 25^{o}C, but the fluid was simply broken beforehand, then tested on the rheometer for a short period of time exposed to the rheometer oven, evaporation was a significant issue.

Two runs were performed for each trial (i.e., one replicate). In most of the analyses presented, the results shown have been averaged between the two trials. In some analyses (i.e., statistical modeling), an "average viscosity" was determined for each trial by taking the average of the viscosities at each shear rate or frequency, depending on the test, at a specific time interval. This simplified analyses greatly, as viscosity was no longer a function of shear rate or frequency, but rather time. Averages were taken from 1-100 radians/second or 1-100 s^{-1}, as these were the ranges where the fluid viscosities had noticeable disparities. At low frequencies and shear rates, all fluids were observed to have similar viscosities.

Filter-Degradation Studies

A key performance parameter of a breaker is its ability to degrade the polymer from a long, possibly networked or crosslinked chain, to smaller oligomers to decrease molecular weight. Residue reduction is important, as well, to maintain well efficiency. Testing was accomplished through a filter-cake filtration method.

Samples were broken similarly to the method described in the previous section, Rheological Studies, although with only one time interval, 18 hours. This time was chosen so that any fluids were broken to their full extent; in other words, no further breaking reaction was expected to occur for any longer than 18 hours. This was determined from preliminary testing. A known volume of sample (7 mL ammonium persulfate, 10 mL breaker) was then added to a Buchner funnel vacuum filtration setup. This can be shown in Figure 12, below. An aspirator was attached to a laboratory water faucet to generate a vacuum in a filter flask. The Buchner funnel was 9 cm in diameter, with Whatman Size 4 filters (20-25 μm, 37 sec/100 mL Herzberg speed). Samples were vacuum filtered for 3 minutes each, with intermediate mixing and washing of the fluid residing on top of the filter with distilled water. Some water and all small oligomers were expected to pass through the filter, leaving only large chains and residual water (e.g., on the filter). Prior to each run, the filter was weighed, then wetted down with distilled water.

Figure 12: Vacuum filtration setup.

To drive off any residual water, to leave only the long chain polymers, each filter (with fluid) was transferred into a pre-weighed foil cup, then placed in a vacuum oven ($80^{o}C$, 0.96 bar) for 24 hours. Following drying, each sample (foil, filter, residue) was weighed. Knowing how much polymer in solution was added to each filter and determining the residue mass, percent permeate, the amount that was able to pass through the filter, was calculated. Two runs were performed for each of the breaker, fluid, concentration factorial experiments. Results were averaged.

Statistical Analyses and Modeling

The statistical analysis software, JMP (SAS Institute) was used to test the statistical of the obtained results. All trials (non-averaged) were input into the software for the three tests: filter-degradation, oscillatory frequency sweep, and steady shear rate sweep. For each, the Fit Model platform was used as follows.

For the two rheological tests, time, fluid, breaker, and concentration were defined as model effects and average viscosity was defined as the model response. Time was defined as numeric and nominal (rather than continuous), fluid was defined as character and nominal (guar or crosslinked guar), as was breaker (each breaker, plus control). Concentration was modeled as character and nominal (low or high) and average viscosity was modeled as numeric and continuous and coded appropriately. This allowed a model to be developed that was more suited for screening effects, rather than a rigorous model for prediction. The nature of this study was for evaluation, rather than mathematical modeling of possible variables. Models were built and refined, beginning with all effects and all cross-effects, for a full-factorial, until an acceptable model was reached and outliers were removed.

For the filter testing, similar methodology was used. Fluid, breaker, and concentration were modeled as effects, with permeate percent the model response. Effects were defined as above, for similar reasoning, and permeate percent was defined as numeric and continuous and coded appropriately. The full-factorial screening model was built and refined, as above, and any outliers were removed.

Key statistical tests performed were whole-test ANOVAs (analysis of variances), effects tests, and Tukey-Kramer pairwise difference tests. Given that the ANOVA and effects tests determined that an effect had a significant effect on the response, Tukey-Kramer tests were particularly helpful in determining which effects (and cross-effects) were not statistically different from one another. Thus, the effects of the enzymatic breakers, for example, could be compared to those of ammonium persulfate. All statistical tests were performed at a significance level of $\alpha = 0.05$.

Economic Analyses

The current market price of each of the three breakers in industrial quantities were determined from the e-commerce company, Alibaba. Several sources/vendors were compared and an average price was taken. Calculations converted all prices to comparable and practical units, the cost to break a set volume of fracking fluid.

Hazard Analyses

Breaker toxicities were evaluated by a literature search of Safety Data Sheets (SDS). Enzymes, by their nature, are regarded as biologically-safe and nontoxic.

Results and Discussion

Rheological Studies

The plots of viscosity over time, for the frequency sweep and steady shear rate sweep tests, on guar and crosslinked guar, are presented below, in Figures 13-16.

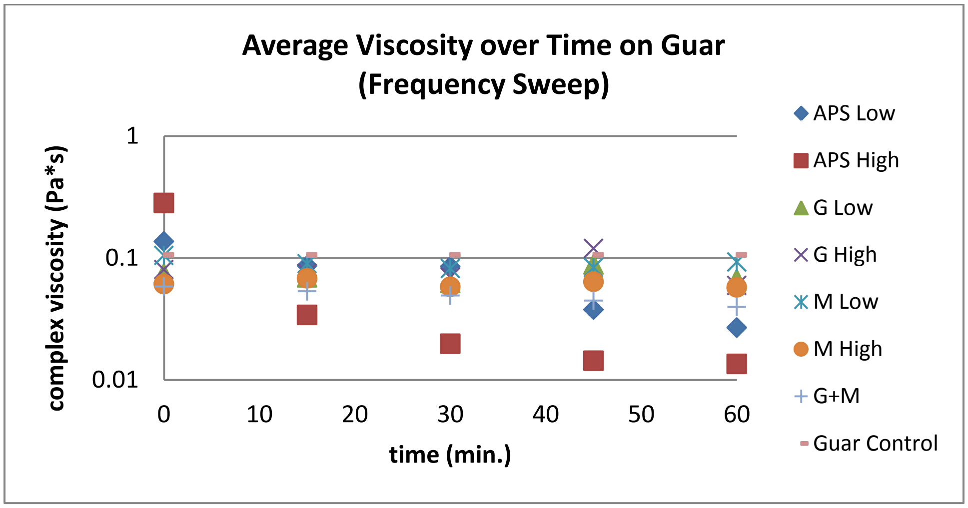

Figure 13: Average viscosity over time on guar, frequency sweep test.

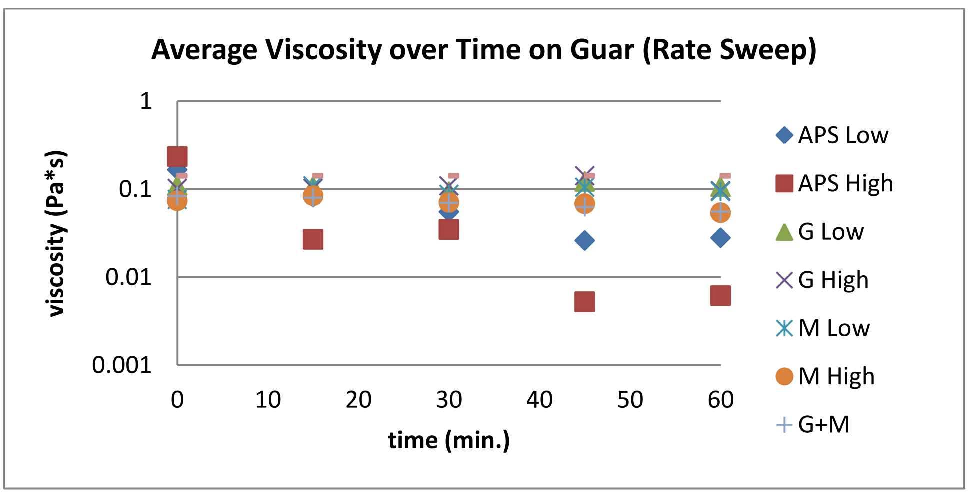

Figure 14: Average viscosity over time on guar, rate sweep test.

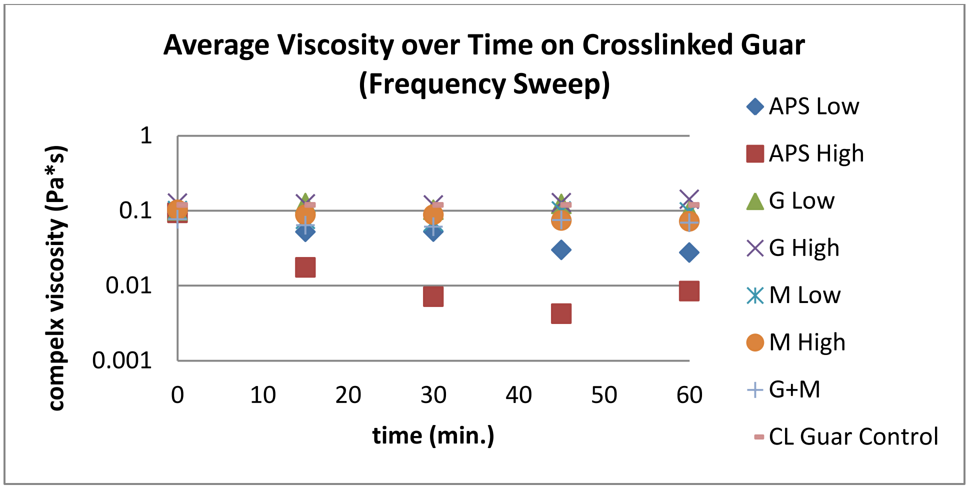

Figure 15: Average viscosity over time on crosslinked guar, frequency sweep test.

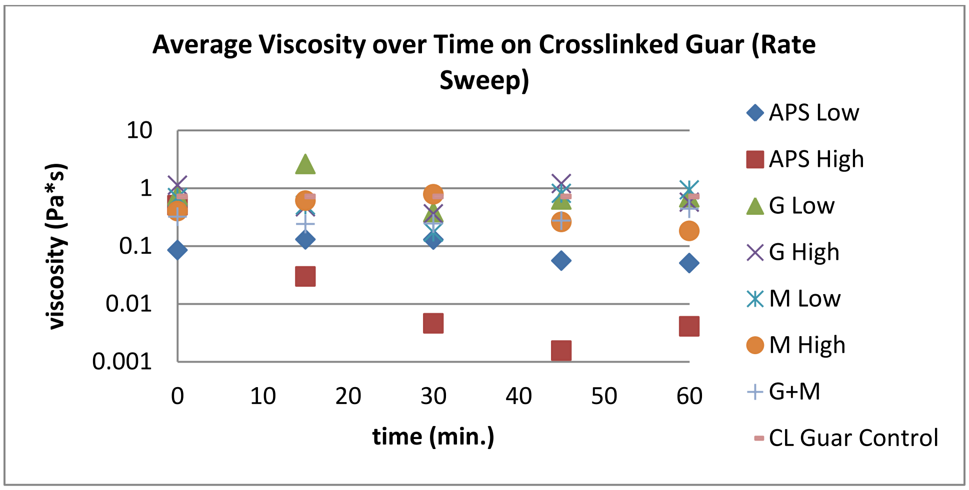

Figure 16: Average viscosity over time on crosslinked guar, rate sweep test.

Plots for rheological tests can be found in Appendix B. Figures B1-B8 show ammonium persulfate, on guar and crosslinked guar at two concentrations; Figures B9-B16 show mannanase, on guar and crosslinked guar, at two concentrations; Figures B17-B24 show galactosidase, on guar and crosslinked guar at two concentrations; Figures B25-28 show the enzyme mix, on guar and crosslinked guar, at one concentration. The data table for average viscosity results obtained from rheological testing can be found in Appendix C, in Table C1.

It can be seen that, as expected, ammonium persulfate is able to substantially decrease the viscosity of the fluid over time. The higher concentration (0.1% wt.) appears able to more rapidly decrease the viscosity than the lower concentration (0.01% wt.). The lower concentration acts within 30-45 minutes and to its full extent by 18 hours, while the higher concentration begins to act within 15 minutes and to its full extent by 1 hour. Similar trends were seen in both of the fluids.

There was not an obvious viscosity change caused by any of the three enzymes tested (mannanase, galactosidase, the mix) on any fluid or at any concentration. If there were any effects from an enzyme, they were not to the same extent as ammonium persulfate. It is shown that there is not much deviation from the control viscosity, either. Statistical analyses will be performed in a future section, Statistical Analyses and Modeling, which will better evaluate breaker comparison.

It is important to note that the results from the 18 hour breaker test were omitted in Figures 13-16, above; however, full results, including 18 hours, can be seen in the rheological plots in Appendix B. Results were omitted because there was noticeable evaporation over this long time period, which impacted the viscosity of the fluid greatly, increasing it by an order of magnitude in some cases compared to the previous time-step, 1 hour. Attempts were made to prevent this evaporation for the filter studies, where fluids were broken 18 hours, by tightly sealing vials.

Filter-Degradation Studies

The plot of average permeate percent, on both guar and crosslinked guar, is shown below, in Figure 17. Blue represents guar fluid; red represents crosslinked. See Appendix C, Tables C2 and C3, for the numerical results of the filter study.

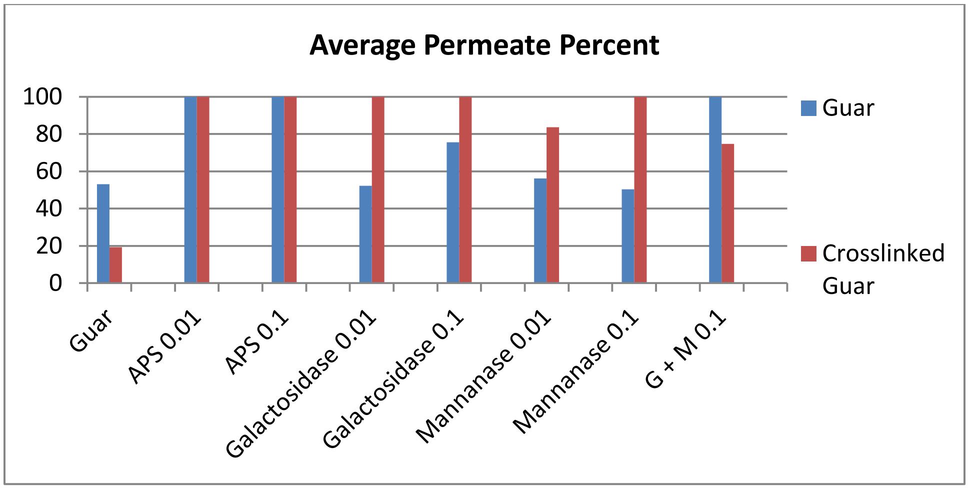

Figure 17: Average filter permeate percent of broken fluids on guar and crosslinked guar.

From Figure 17, it is shown that, on average, 53% of guar can pass through the filter, and 19.2% of the crosslinked guar can pass through the filter without breaking. As expected, for both fluids, the ammonium persulfate is able to degrade the polymer enough to allow it to completely pass through the filter. For guar, the enzyme mix of galactosidase and mannanase is able to do this, as well. However, for mannanase and galactosidase separately, it does not appear the enzymes are able to function. Only about 50% of the enzymes samples, at either concentration, can permeate the filter. This is not much different from the control.

The crosslinked fluid results are, at least at first glance, confounding. It would be expected that less of these samples are able to permeate through the filter, as they are highly-networked and viscous. The control explains this, as not much can pass through. However, nearly every fluid but the mannanase and galactosidase-mannanase enzyme mix is capable of degrading the fluid to the extent required for it to pass completely through the filter. Some of this may be due to experimental error or methodology (to be explained), but there may be merit to these results.

The galactosidase, in cleaving off its substrate side chains, could be predicted to cleave off some of the galactose units native to the guar structure, as well as some of the galactose units that have crosslinked with other chains. Thus, galactosidase should not be able to decrease the viscosity of the crosslinked guar much more than that of guar, as the most it is able to degrade the crosslinked fluid is to cleave crosslinks and side chains. However, even though the viscosity has not been substantially decreased because there remains an abundance of high molecular weight chains in the fluid, it is possible that it has been degraded enough so that it can pass through a filter. Orientation and dilution with water could play a key role here: a chain that has few to no side chains may be able to quite easily pass through the filter length-wise, especially if able to easily move within solution. Mannanase is able to degrade the guar, as well, to a high-extent, though at the lower concentration, not completely. This, along with the enzymatic mixture (consisting of high concentrations of galactosidase and mannanase) cannot be easily explained, especially because these two enzymes worked well individually.

Some nonidealities in the data may be attributed to a large filter size. The filter used was quite large and the grade was designated as less analytical than was desired. Tuning filter size to the system and application may increase resolution and allow the possibility for more concrete conclusions to be made from filtration.

Additionally, an odd phenomenon was observed in calculations. In some tests, a sample had a negative mass after being filtered and oven-dried. This was likely due to losses in mass from fluid transferring and paper volatiles flashing off upon heating. In the case that a negative mass was found, it was corrected to zero. While this likely produced some inaccuracy in results, any sample that had an observed negative mass had much of the sample permeate, regardless. Changes in mass in these cases were quite small and were only marginal amounts (from transferring, loss of volatiles, etc.). Note that these effects were likely compounded by the volumes (and therefore masses) of samples that were added to the filters being so small. Thus, small changes had significant effects on results.

Statistical analyses will be performed in the next section, Statistical Analyses and Modeling. Here, breaker comparison from results will be extensively evaluated.

Statistical Analyses and Modeling

From the three distinct tests performed, three statistical models were constructed. The model for the oscillatory frequency sweep test results are shown below, in Figures 18-21. Figure 18 shows the summary of fit and ANOVA results. Figure 19 shows the effects tests, while Figures 20-21 show the Tukey-Kramer analyses.

Summary of Fit

RSquare	0.677893
RSquare Adj	0.655245
Root Mean Square Error	0.020894
Mean of Response	0.071033
Observations (or Sum Wgts)	138

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	0.11759784	0.013066	29.9315
Error	128	0.05587770	0.000437	Prob > F
C. Total	137	0.17347555		<.0001*

Figure 18: Summary of fit and ANOVA results for frequency sweep model.

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Fluid	1	1	0.00747617	17.1258	<.0001*
Breaker	4	4	0.08891352	50.9189	<.0001*
Fluid*Breaker	4	4	0.01210849	6.9343	<.0001*

Figure 19: Effects tests for frequency sweep model.

Level					Least Sq Mean
None	A				0.11273333
G	A				0.09550408
M		B			0.07894395
G+M			C		0.05919000
APS				D	0.03265625

Levels not connected by same letter are significantly different.

Figure 20: Tukey-Kramer report for breaker of frequency sweep model.

Level						Least Sq Mean
CL Guar,None	A	B				0.11953333
CL Guar,G	A					0.11924500
Guar,None	A	B	C			0.10593333
CL Guar,M		B	C			0.08703000
Guar,G			C	D		0.07176316
Guar,M			C	D		0.07085789
CL Guar,G+M			C	D		0.06933000
Guar,G+M				D	E	0.04905000
Guar,APS					E	0.03426250
CL Guar,APS					E	0.03105000

Levels not connected by same letter are significantly different.

Figure 21: Tukey-Kramer report for breaker/fluid cross of frequency sweep model.

Overall, the oscillatory frequency sweep model is acceptable. Its R^2 value is approximately 0.68, meaning that it accounts for 68% of the variability in results. The whole-test ANOVA has a p-value of < 0.0001, so at least one of the effects had a significant effect on the viscosity. The effects test shows which effects. In this case, fluid, breaker, and the cross-effect of fluid and breaker had an effect (all $p < 0.0001$). Neither time nor concentration had a statistically-significant effect.

As fluid had an effect, the Tukey-Kramer test is redundant; guar and crosslinked guar had statistically-different effects on viscosity. For breaker, there was no enzyme that was not statistically-different from ammonium persulfate. Galactosidase, in fact, did not have an effect that was statistically-different from adding no breaker at all. However, in the Tukey-Kramer test, the cross-effect of the enzyme mixture on guar is not statistically-different from that of ammonium persulfate (ammonium persulfate on both fluids were never statistically-different).

The model for the steady shear rate sweep test results are shown below, in Figures 22-25. Figure 22 shows the summary of fit and ANOVA results. Figure 23 shows the effects tests, while Figures 24 and 25 present the Tukey-Kramer test analyses.

Summary of Fit

RSquare	0.893995
RSquare Adj	0.885322
Root Mean Square Error	0.045868
Mean of Response	0.141525
Observations (or Sum Wgts)	120

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	1.9517403	0.216860	103.0763
Error	110	0.2314266	0.002104	Prob > F
C. Total	119	2.1831668		<.0001*

Figure 22: Summary of fit and ANOVA results for rate sweep model.

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Breaker	4	4	1.0205408	121.2690	<.0001*
Fluid	1	1	0.9188094	436.7218	<.0001*
Fluid*Breaker	4	4	0.5123674	60.8837	<.0001*

Figure 23: Effects tests for rate sweep model.

Level					Least Sq Mean
None	A				0.46678333
G		B			0.25874932
M			C		0.17581500
G+M			C		0.16463222
APS				D	0.04762500

Levels not connected by same letter are significantly different.

Figure 24: Tukey-Kramer report for breaker of rate sweep model.

Level							Least Sq Mean
CL Guar,None	A						0.79140000
CL Guar,G		B					0.40816364
CL Guar,M			C				0.26923000
CL Guar,G+M			C				0.25884444
Guar,None				D	E		0.14216667
Guar,G				D			0.10933500
Guar,M				D	E	F	0.08240000
Guar,G+M				D	E	F	0.07042000
CL Guar,APS					E	F	0.05462778
Guar,APS						F	0.04062222

Levels not connected by same letter are significantly different.

Figure 25: Tukey-Kramer report for breaker/fluid cross of rate sweep model.

Overall, the oscillatory frequency sweep model is good. Its R^2 value is approximately 0.90, meaning that it accounts for 90% of the variability in results. The whole-test ANOVA has a p-value of < 0.0001, so at least one of the effects had a significant effect on the viscosity. The effects test shows which effects. In this case, fluid, breaker, and the cross-effect of fluid and breaker had an effect (all $p < 0.0001$). Neither time nor concentration had a statistically-significant effect.

As fluid had an effect, the Tukey-Kramer test is redundant; guar and crosslinked guar had statistically-different effects on viscosity. For breaker, there was no enzyme that was not statistically-different from ammonium persulfate. However, in the Tukey-Kramer test, the effect of the enzyme mixture on guar and the effect of mannanase on guar were not statistically-different from ammonium persulfate.

The model from the filter-degradation test results are shown below, in Figures 26-29. Figure 26 shows the summary of fit and ANOVA results. Figure 27 shows the effects tests, while Figures 28 and 29 present the Tukey-Kramer test analyses.

Summary of Fit

RSquare	0.952585
RSquare Adj	0.932265
Root Mean Square Error	6.857275
Mean of Response	78.60645
Observations (or Sum Wgts)	31

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	19838.732	2204.30	46.8779
Error	21	987.467	47.02	Prob > F
C. Total	30	20826.199		<.0001*

Figure 26: Summary of fit and ANOVA results for filter model.

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Fluid	1	1	179.572	3.8189	0.0641
Breaker	4	4	11470.455	60.9842	<.0001*
Fluid*Breaker	4	4	7136.348	37.9414	<.0001*

Figure 27: Effects tests for filter model.

Level		Least Sq Mean
APS	A	100.00000
G+M	B	87.35000
G	B C	77.06667
M	C	72.55000
None	D	36.15000

Levels not connected by same letter are significantly different.

Figure 28: Tukey-Kramer report for breaker of filter model.

Level		Least Sq Mean
CL Guar,APS	A	100.00000
CL Guar,G	A	100.00000
Guar,APS	A	100.00000
Guar,G+M	A	100.00000
CL Guar,M	A B	91.80000
CL Guar,G+M	B C	74.70000
Guar,G	C D	54.13333
Guar,M	D	53.30000
Guar,None	C D	53.00000
CL Guar,None	E	19.30000

Levels not connected by same letter are significantly different.

Figure 29: Tukey-Kramer report for breaker/fluid cross of filter model.

Overall, the oscillatory frequency sweep model is good. Its R^2 value is approximately 0.95, meaning that it accounts for 95% of the variability in results.

The whole-test ANOVA has a p-value of < 0.0001, so at least one of the effects had a significant effect on the viscosity. The effects test shows which effects. In this case, breaker and the cross-effect of fluid and breaker had an effect ($p < 0.0001$). Neither time nor concentration had a statistically-significant effect. Fluid did not have an effect, either, but was retained to have a hierarchal model.

As fluid had an effect, the Tukey-Kramer test is redundant; guar and crosslinked guar had statistically-different effects on viscosity. For breaker, there was no enzyme that was not statistically-different from ammonium persulfate. However, in the Tukey-Kramer test, the effect of galactosidase on crosslinked guar, the enzyme mixture on guar, and mannanase on crosslinked guar were not statistically different from the effects of ammonium persulfate on guar or crosslinked guar.

From these analyses, it is clear that there is are no enzymatic breakers that are capable of competing with ammonium persulfate. In each of the three models constructed, the effects of enzymatic breakers were statistically-different from those of ammonium persulfate. However, enzymes do show some promise. The enzymes have some function, as there are some non-different cross-effects of fluid and breaker among ammonium persulfate and the enzymes. Additionally, in most cases, the enzymes are statistically-different from the controls (no breaker added). Thus, the enzymatic breakers work, but not to the desired, full extent.

It is important that future testing includes more replicates. While replicates are always essential for ensuring repeatability of experimental results, constructing such a large model (up to five effects, with a full-factorial of cross-effects) requires many observations. A full-factorial model, while likely not realistic, was not possible to construct here, as the limited observations resulted in some losses of degrees of freedom and bias in statistical analyses. With the obtained results to move forward with, tests can now be better designed to eliminate these issues.

In none of the models, time and concentration had statistically-significant effects. One explanation is that these effects were simply masked by effects with "more significant" impacts on responses, especially with the restrictive sample size. The other is that they simply did not have practically-significant effects. On small time-scales, with only several time-steps, this may not be surprising, especially considering that, if a breaker was to work, it typically acted quickly. Additionally, the enzymatic breakers are catalytic and the ammonium persulfate starts a chain reaction, so concentration could be expected not to have any effect, as observed.

Economic Analyses

The current market prices of the breakers in question are shown below, in Table 2.

Table 2: Market prices of bulk breaker chemicals (as of March 22, 2016) **[30]**.

Breaker	**Current Market Price**
Ammonium Persulfate	$0.72/kg
Mannanase	$9/kg (20,000 U/g)
Galactosidase	$35/kg (1,000 U/g)

In Table 3, below, the cost of these breakers to treat a volume of fluid are shown.

Table 3: Breaker costs to treat specified volumes of fracking fluid.

Breaker	Concentration	Cost ($/m^3 fluid)	Cost ($/1,000 gal fluid)
Ammonium Persulfate	0.01% wt.	0.07	0.27
	0.1% wt.	0.72	2.73
Mannanase	0.01 U/mL	0.005	0.02
	0.1 U/mL	0.05	0.17
Galactosidase	0.01 U/mL	0.35	1.32
	0.1 U/mL	3.50	13.25
Mannanase + Galactosidase	0.1 U/mL	3.55	13.42

If an alternative breaker is to be widely-adopted by industry, it generally cannot be more expensive than what is currently used. Ammonium persulfate is quite inexpensive, as seen in Table 3, but mannanase is less expensive. Galactosidase, however, is significantly more expensive and this results in the two enzymes together being significantly more expensive, as well. Galactosidase would not be a viable breaker, while there are no economic issues with a mannanase breaker.

Hazard Analyses

Reported breaker health and environmental hazards are shown in Table 4, below.

Table 4: Environmental and health hazards of breakers **[31]**.

Breaker	Effects
Ammonium Persulfate	acute toxin (oral LD_{50}: 700 mg/kg, dermal LD_{50}: 2000 mg/kg); eye, skin, and respiratory irritant; immunosuppressant; damage to gastrointestinal tract; acute and chronic aquatic toxin (LC_{50} for fish: 76 mg/L for 96 h)
Enzymes	benign to human health and the environment

Ammonium persulfate has a host of reported toxic effects on human health and the environment. It is an acute toxin, meaning that adverse effects can occur after a single exposure. However, it is unlikely such concentrations will be consumed if groundwater is contaminated; it is more likely that it would cause irritation, damage to the gastrointestinal tract, and decrease immune response. The worst result would likely be environmental contamination. At relatively low concentrations in aquatic ecosystems, for example, the toxicity is lethal to fish populations. Enzymes, on the other hand, do not post significant health hazards.

Conclusion

Summary

Two enzymatic breakers, β-mannanase and α-galactosidase, were proposed as alternatives to ammonium persulfate for hydraulic fracturing ("fracking") applications. While fracking is an important and relevant method for enhanced energy recovery in the United States, many of the components in current fracking fluids are hazardous to human health and the environment and there are fears that fracking fluids may contaminate drinking water and local ecosystems. To reduce the risk associated with fracking, more environmentally-friendly fracking fluids can be used. Ammonium persulfate is a potent toxin to humans and the environment, while enzymes are biologically-benign. However, for adoption in industry, these enzymes must function as well or better as ammonium persulfate as a breaker. Rheological, filter-degradation, economic, and hazard analyses were performed to compare these breakers, in search of a better and safer alternative.

Significance & Recommendations

It was found that, through comprehensive and extensive testing and research, the two proposed breakers, β-mannanase and α-galactosidase, and a mix of the two enzymes, cannot match the performance of ammonium persulfate. Through testing rheological and residue reduction properties of the fluids, ammonium persulfate is able to much more effectively reduce viscosity and better degrade the fluid. Tests were validated by statistical analyses. This is disconcerting, as the health and environmental hazardousness of ammonium persulfate were analyzed. If a loss event (i.e., contamination) took place, it is likely that ammonium persulfate could cause significant damage to local communities and ecosystems.

Galactosidase, as could be expected, does not perform well individually as a breaker. It, at most, can cleave side chains of the guar polymer and undo crosslinks between chains in the crosslinked fluid. Mannanase seems to have some beneficial effect in reducing viscosity and residue; however, it cannot match the performance of ammonium persulfate. In theory, its mechanism in breaking the main chain does have some promise of having the desired effect and the results of this experiment confirm this. Mannanase has some effect on reducing viscosity and residue. The mixture of mannanase and galactosidase shows the most promise in replacing ammonium persulfate (with mannanase likely the more contributing component), although it still cannot compete with ammonium persulfate. It is worth noting that, even if the mixture were determined to be able to replace ammonium persulfate in terms of rheological and residue reduction properties, it is not feasible for industrial use. The galactosidase and mannanase mixture costs approximately 50 times more than the lowest concentration of ammonium persulfate to treat the same volume of fracking fluid. Here, galactosidase is the largest contributor toward cost. Mannanase is actually about 14 times less expensive than ammonium persulfate at similar concentration levels.

Despite the lack of success in determining a suitable alternative for ammonium persulfate, this work must continue. Fracking will likely continue, regardless of its risks, and to mitigate this, more environmentally-friendly fluid components should be found. More than that, however, it has been found that enzymes have some potential to replace ammonium persulfate and other oxidative breakers. With this knowledge and these results, future experimentation may move forward.

Contribution

This work is not entirely novel. As described in the Literature Review, above, much work has been performed in the area of enzymatic breakers for fracking applications. However, this work considers new areas that have not been previously explored and adds significant research findings to existing knowledge.

Foremost, much of the work done previously has been proprietary. Companies that have developed engineered enzymes for fracking applications publish their findings, but are purposefully vague and sparse in describing their work, even going to far as to not disclose the chemical identity or nature of their new breaker. Much of the work is published in trade journals, such as the *Society of Petroleum Engineers*. One motivation of this work was to validate claims that such enzymatic breakers are relevant and can compete with conventional breakers.

As a part of this, industrial-grade enzymes were used for testing, rather than engineered ones. This eliminated the secrecy required by petroleum companies in disclosing results, intellectual property constraints, and allowed for an independent evaluation of "native" enzymes for breaking potential. The goal of the work was not to promote or advertise any particular enzyme; it was to be purely objective and evaluate breakers based solely on performance. While engineered enzymes would likely add benefit for field use, the evaluation of "native" enzymes is quite novel and provided true judgment of breaker abilities.

Additionally, cost and hazard analyses are usually not included in such publications and have not been seen in previous work. Cost analyses are simply not examined in many cases, especially in trade journals advocating for a specific breaker or in papers written by vendors or petroleum companies. Hazard analyses are not, either. While environmental and health concerns are likely considered, they are not typically reported (unless mandated). This is of particular concern, as many of the companies publishing and selling enzymatic breakers are vendors of ammonium persulfate and other oxidative breakers, as well. This is a clear conflict of interest and hazard analyses are likely not reported with fidelity. Generally, the environmental and health perspective of this work is quite novel.

Some aspects of testing are new, as well. Galactosidase, as well as the enzymatic mix, is not typically considered as an enzymatic breaker. Typical enzymatic breakers are mannanases. The reason for this is quite obvious: the breaking mechanism for galactosidase is predicted to have significantly less of an effect than mannanase. To perform comprehensive testing, however, as well as incorporate testing of a synergistic mixture of enzyme for optimal breaking, galactosidase was considered. The suite of rheological tests are often not performed, either. In most cases, a simple rotational viscometer is used. The results from those studies are simply not as rigorous or accurate as those presented here. The results from this fluid far better describe the fluids' properties. The statistical means of breaker evaluation have not been seen, either. This is likely due to the lacking reporting of results usually published in the subject area. Such statistical methods are the most reliable and definitive way to analyze data.

Future Work

Future testing should focus on several key areas to move forward in identifying alternative breakers. Some areas were identified from this work; others have not yet been considered. Foremost, the testing of "better" enzymes - enzymes that have increased thermo- or pH-stability, activity, or selectivity, from engineering or screening - would be essential for comparing enzymatic vs. oxidative breakers. While this may force a partnership to be developed with a company that produces such enzymes, perhaps leading to reduced freedom in experimentation, a side-by-side comparison of breakers specifically suited for these purposes and applications, those that would actually be used in the field, would be most telling.

It would be ideal if the group could collaborate with another group that is better-suited and has more expertise in protein engineering. Working in an academic setting could prevent many of the downsides from working with an industry partner. However, this would require a great deal of funding, time, and resources.

This work explores only a small region of the design space: 50^oC and atmospheric pressure (1 bar). While 50^oC is the "average" well temperature, fracking wells can range widely in temperature, from $25\text{-}150^oC$, and no fracking well truly operates at atmospheric pressure. Breaker performance at varying conditions have not been explored at all here and knowledge of how these results change would be critical.

If time and concentration truly do not have statistically-significant effects on the results here, then future testing may eliminate them from experimentation - or reduce their importance in designing experiments, and focusing on other effects instead. Note that it is possible that time and concentration did have statistically-significant effects here, they were just not practically-significant and/or were masked by other effects. Future testing may look into these questions, as well.

As a part of this, if concentration does not have a statistically-significant effect, then an interesting experiment would involve determining to what extent the ammonium persulfate concentration can be reduced while maintaining the desired effect. This does deviate from finding a true alternative breaker to ammonium persulfate, but decreasing the amount of ammonium persulfate would have the same result: decreasing the environmental and health hazards associated with the fluid. It would be expected that a concentration would be reached where the reaction could simply not proceed or be sustained. Determining at what point this occurs may have the ability to greatly reduce the toxicity of breakers in these fluids. Extending this idea, it would be interesting to determine how the ammonium persulfate concentration may be reduced (or further reduced, with the above plan) if it is supplemented by an enzymatic breaker, such as the enzyme mix. Studies show oxidative and enzymatic breakers can work cooperatively [23].

A final area of work would be investigating other oxidative breakers for comparison with enzymatic breakers, similar to what was done here with ammonium persulfate. Ammonium persulfate is not the only breaker used in industry. More expansive testing will give further insight into breaker evaluation.

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Appendix A: Enzyme pH and Thermostability Figures

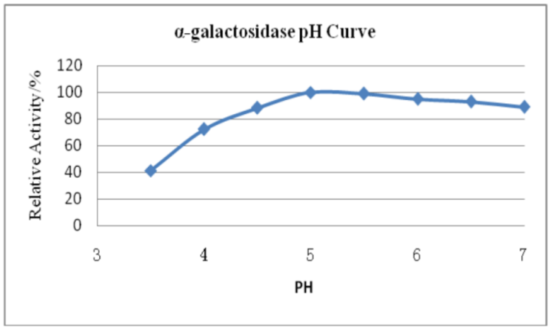

Figure A 1: Galactosidase pH-stability curve **[20]**.

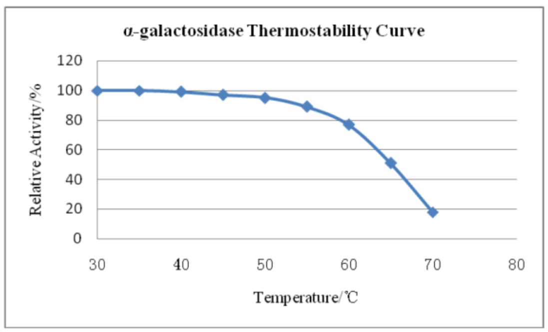

Figure A 2: Galactosidase thermostability curve **[20]**.

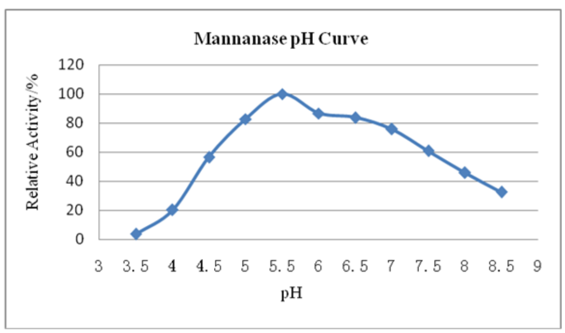

Figure A 3: Mannanase pH-stability curve **[19]**.

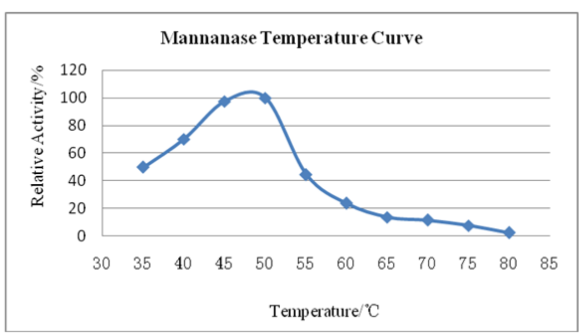

Figure A 4: Mannanase thermostability curve [19]**.**

Appendix B: Additional Rheological Figures

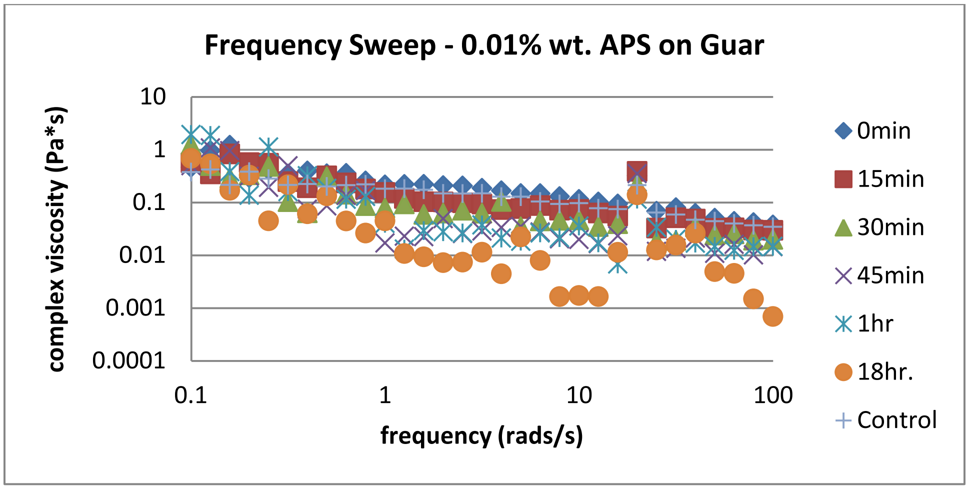

Figure B 1: Frequency sweep for 0.01% wt. ammonium persulfate on guar.

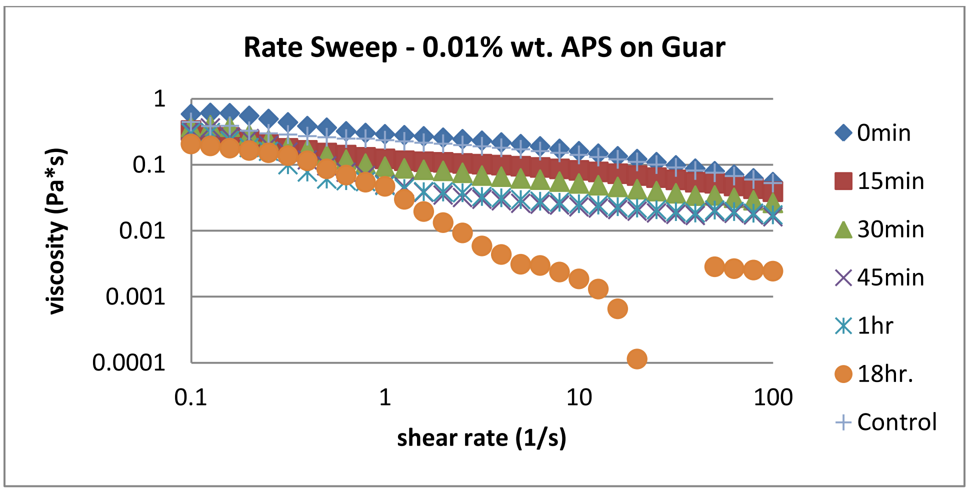

Figure B 2: Rate sweep for 0.01% wt. ammonium persulfate on guar.

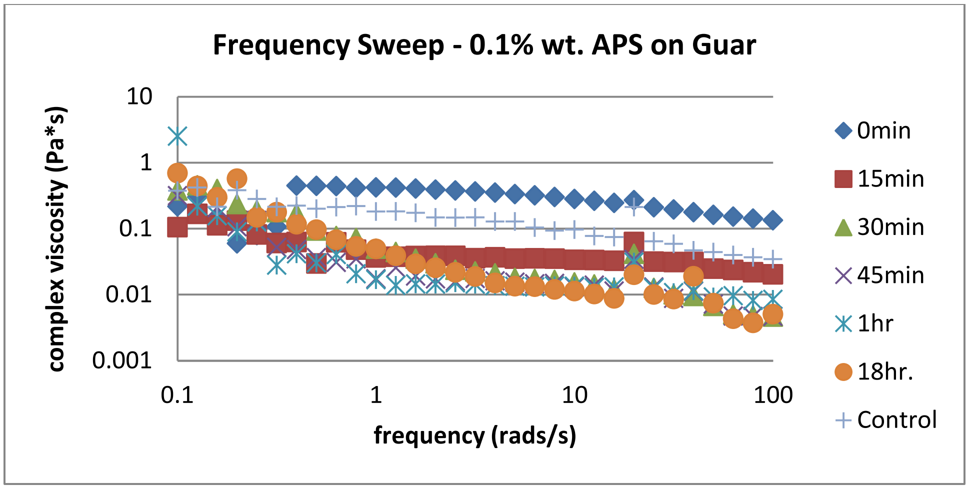

Figure B 3: Frequency sweep for 0.1% wt. ammonium persulfate on guar.

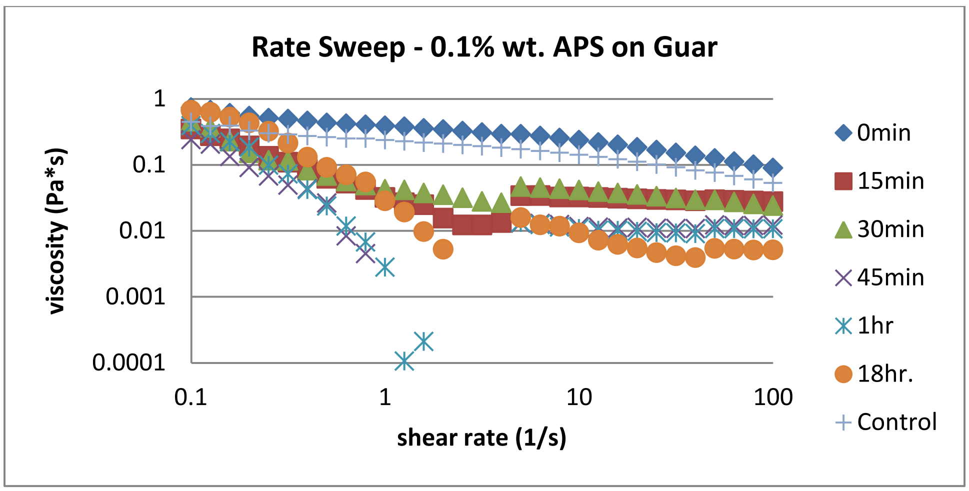

Figure B 4: Rate sweep for 0.1% wt. ammonium persulfate on guar.

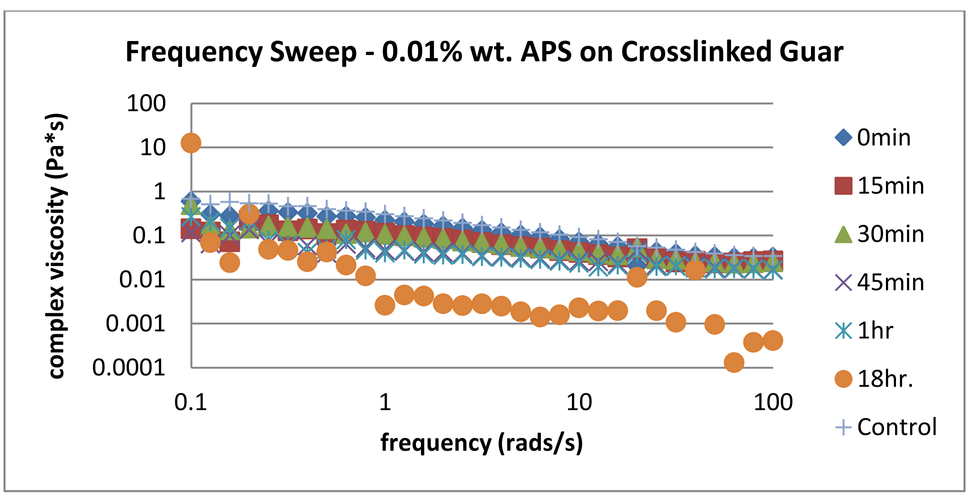

Figure B 5: Rate sweep for 0.01% wt. ammonium persulfate on crosslinked guar.

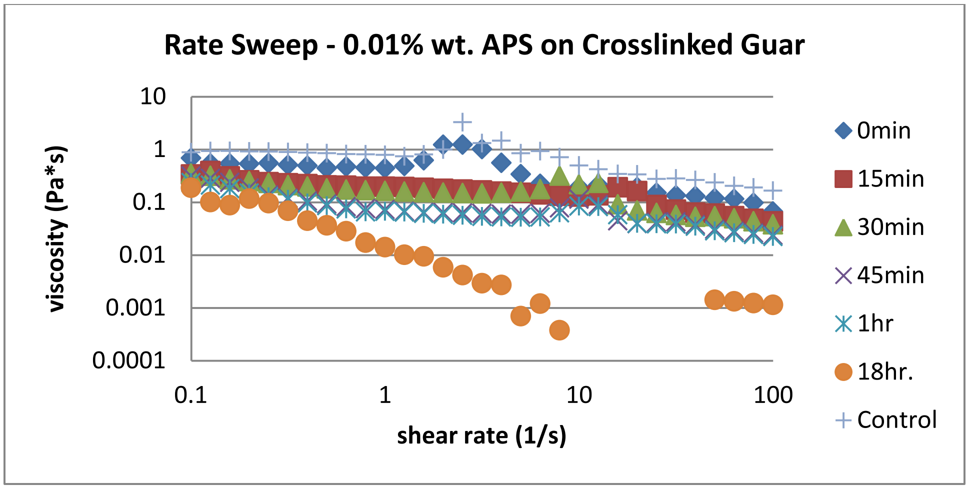

Figure B 6: Rate sweep for 0.01% wt. ammonium persulfate on crosslinked guar.

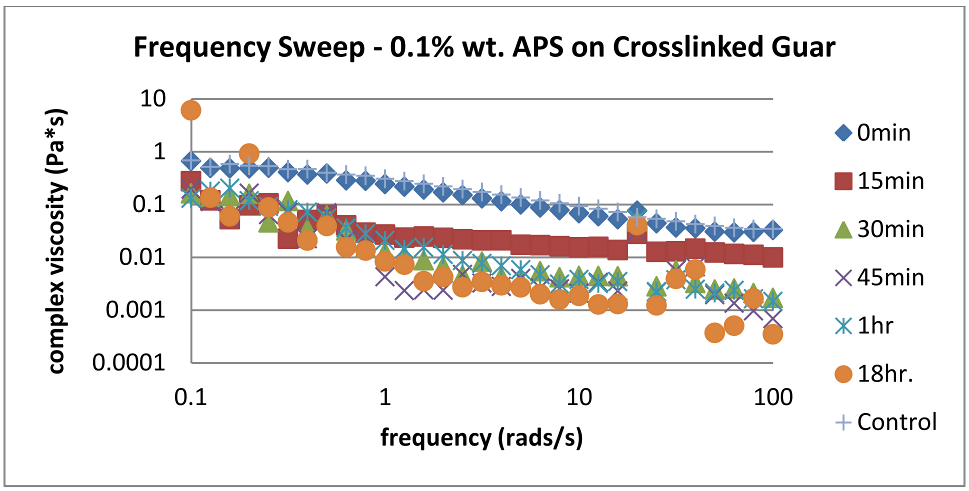

Figure B 7: Frequency sweep for 0.1% wt. ammonium persulfate on crosslinked guar.

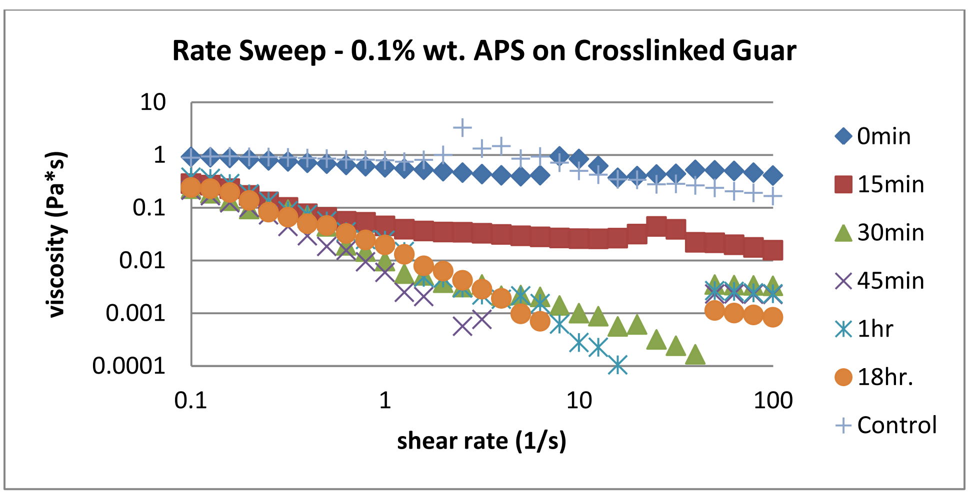

Figure B 8: Rate sweep for 0.1% wt. ammonium persulfate on crosslinked guar.

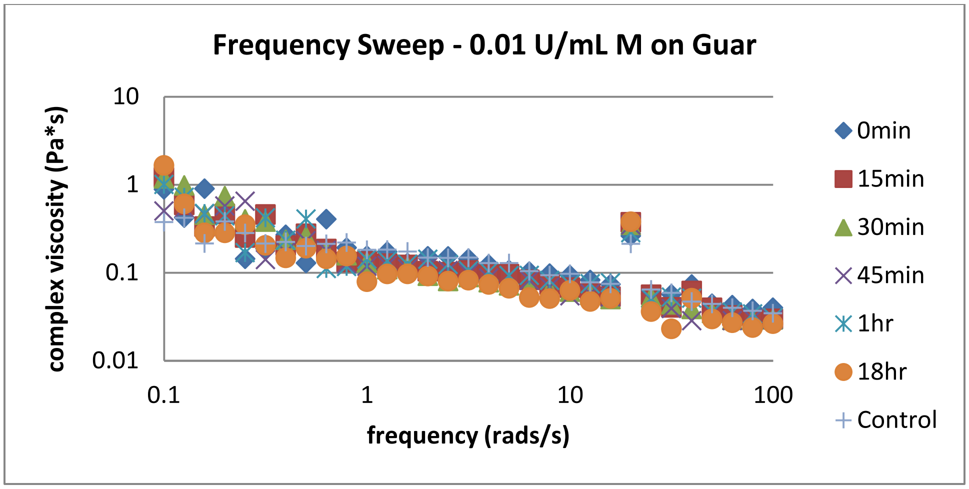

Figure B 9: Frequency sweep for 0.01 U/mL mannanase on guar.

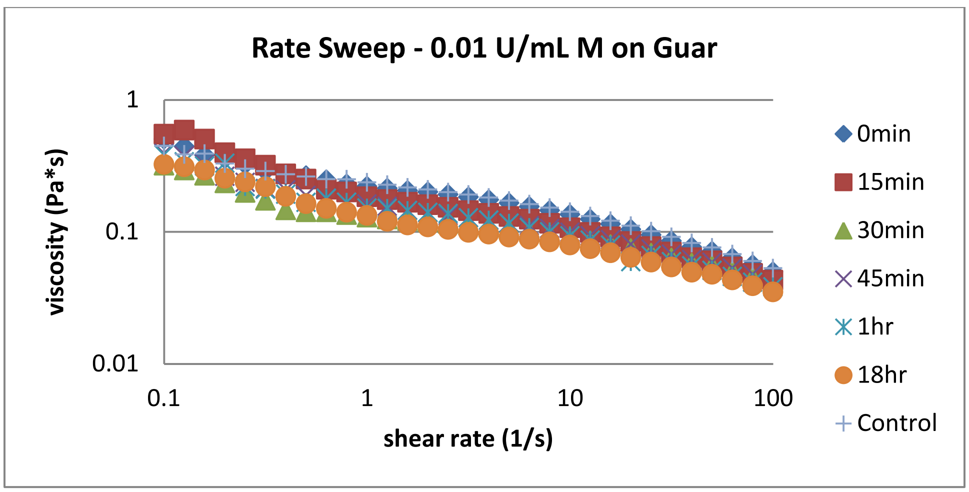

Figure B 10: Rate sweep for 0.01 U/mL mannanase on guar.

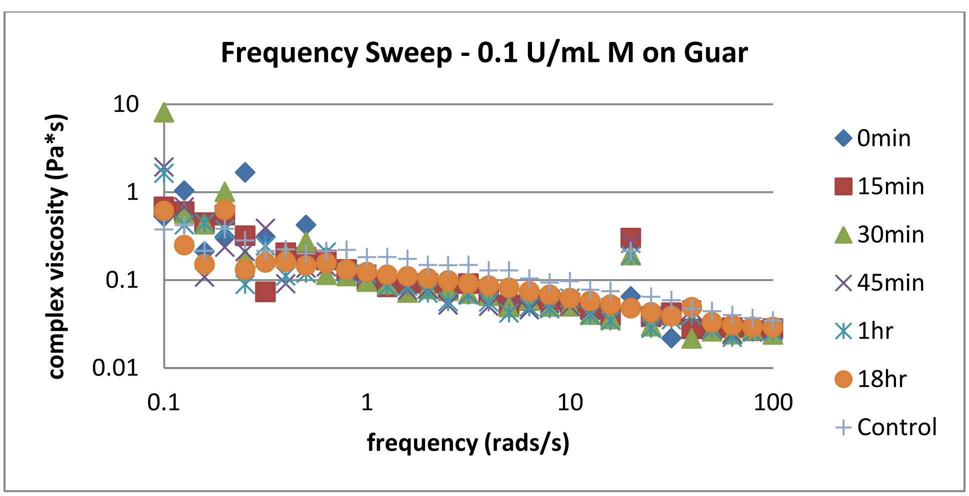

Figure B 11: Frequency sweep for 0.1 U/mL mannanase on guar.

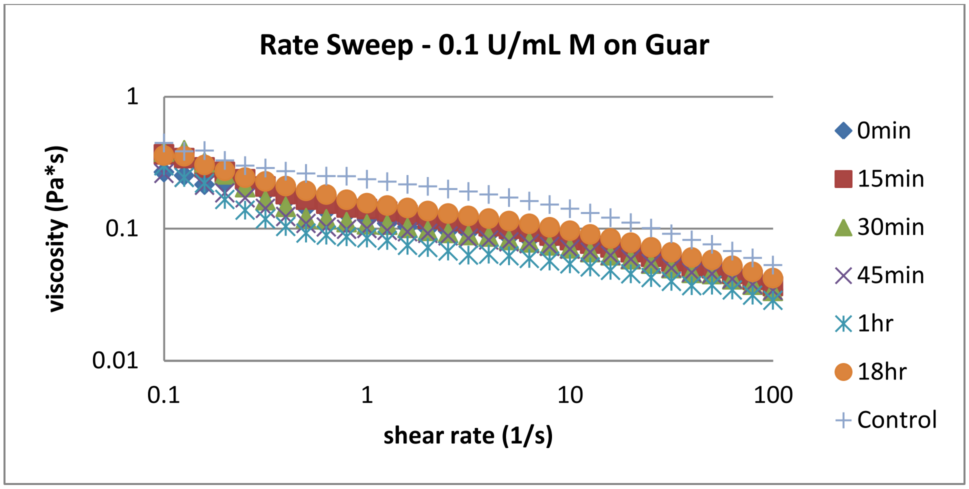

Figure B 12: Rate sweep for 0.1 U/mL mannanase on guar.

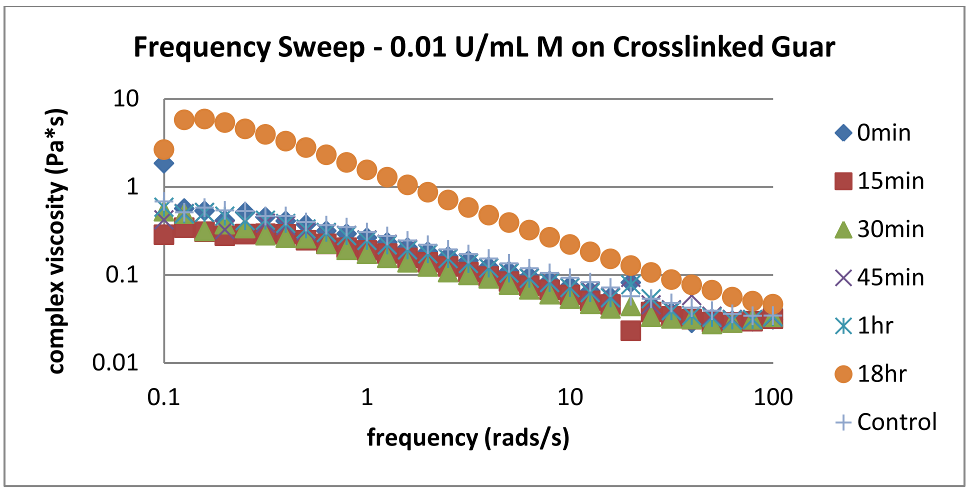

Figure B 13: Frequency sweep for 0.1 U/mL mannanase on crosslinked guar.

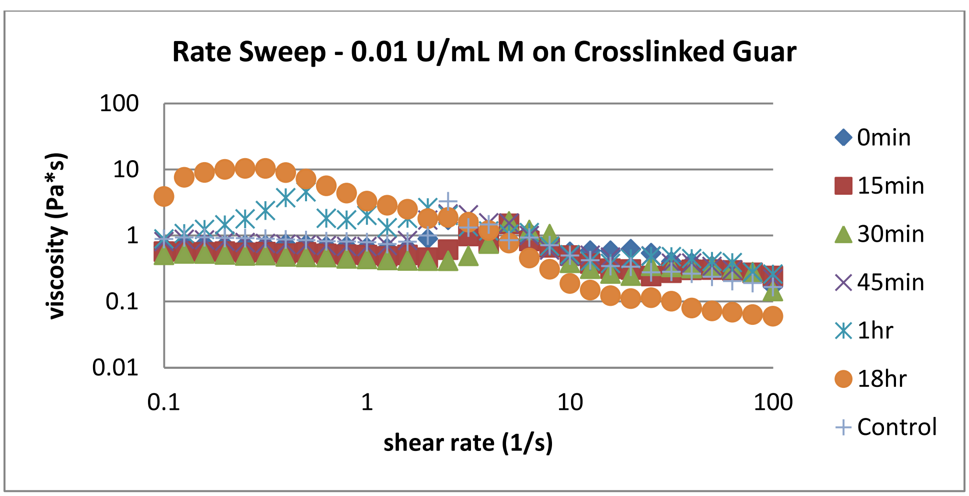

Figure B 14: Rate sweep for 0.1 U/mL mannanase on crosslinked guar.

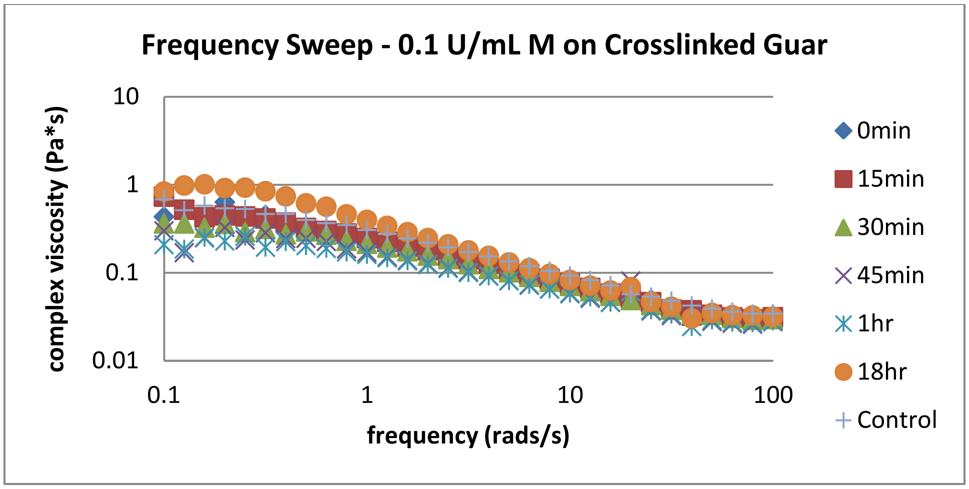

Figure B 15: Frequency sweep for 0.1 U/mL mannanase on crosslinked guar.

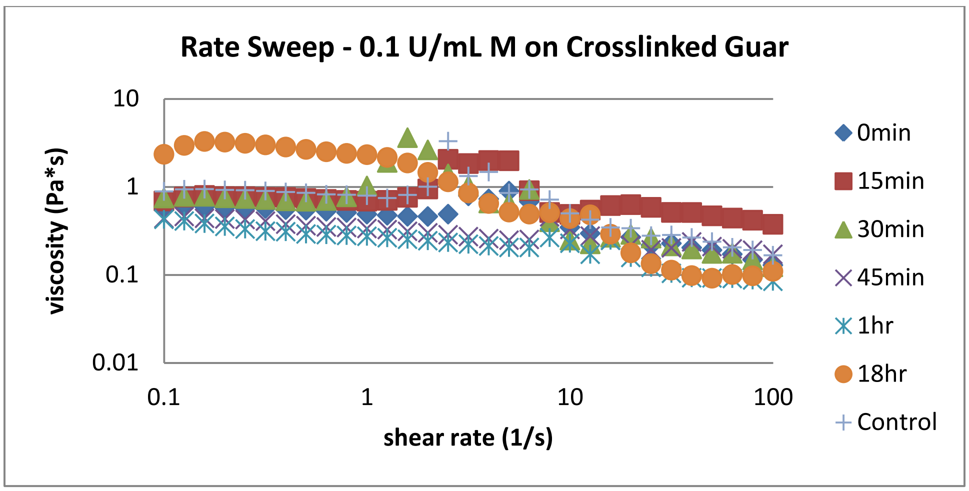

Figure B 16: Rate sweep for 0.1 U/mL mannanase on crosslinked guar.

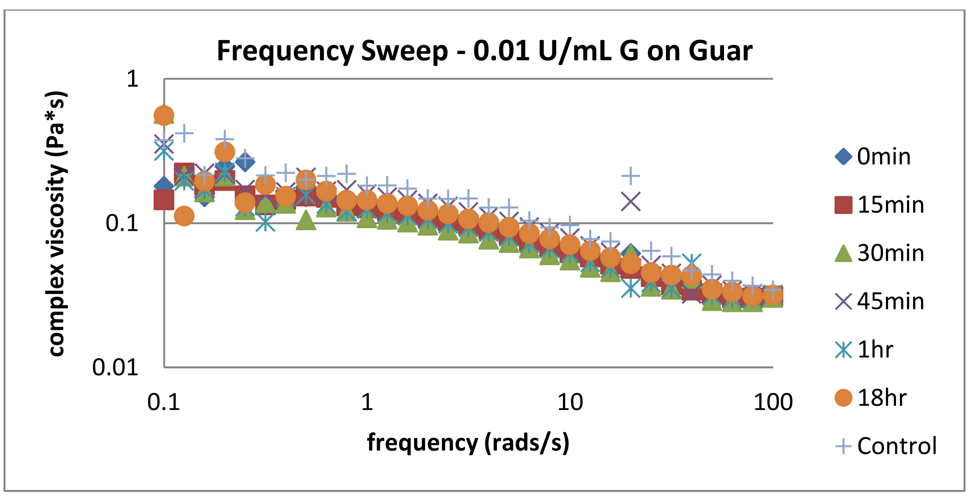

Figure B 17: Frequency sweep for 0.01 U/mL galactosidase on guar.

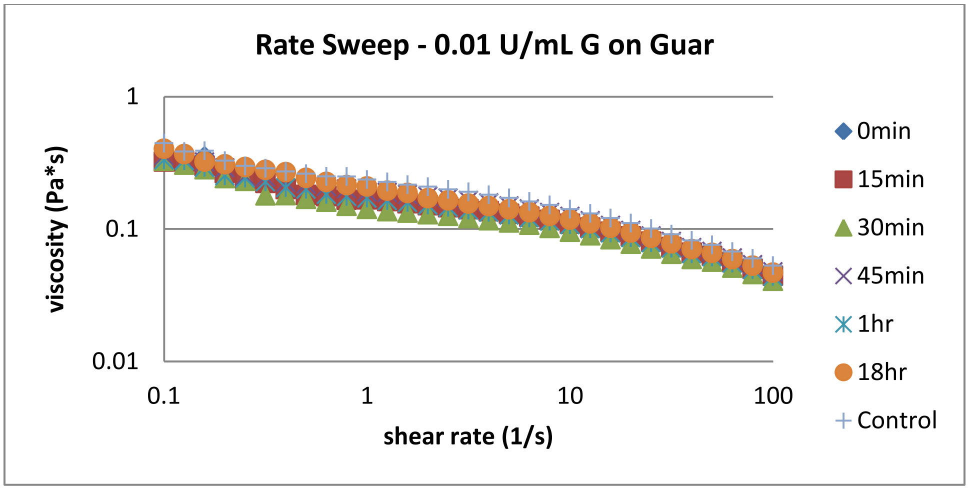

Figure B 18: Rate sweep for 0.01 U/mL galactosidase on guar.

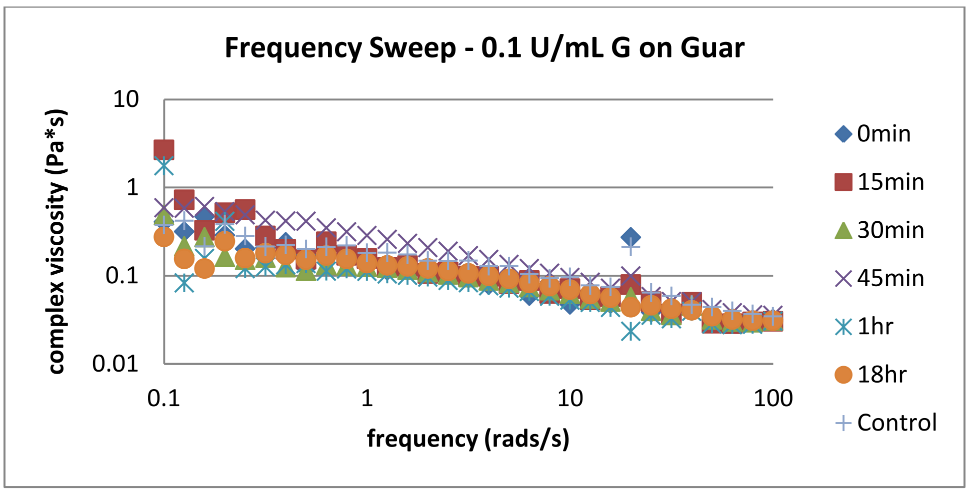

Figure B 19: Frequency sweep for 0.1 U/mL galactosidase on guar.

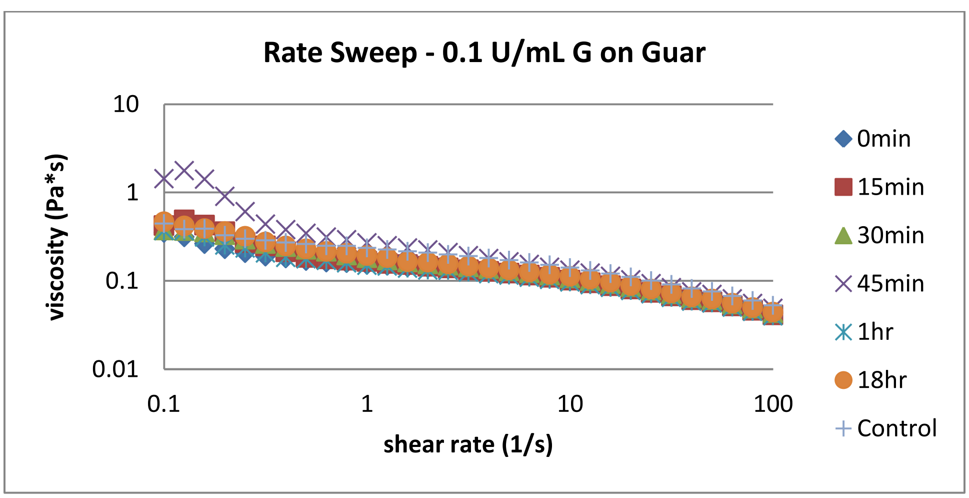

Figure B 20: Rate sweep for 0.1 U/mL galactosidase on guar.

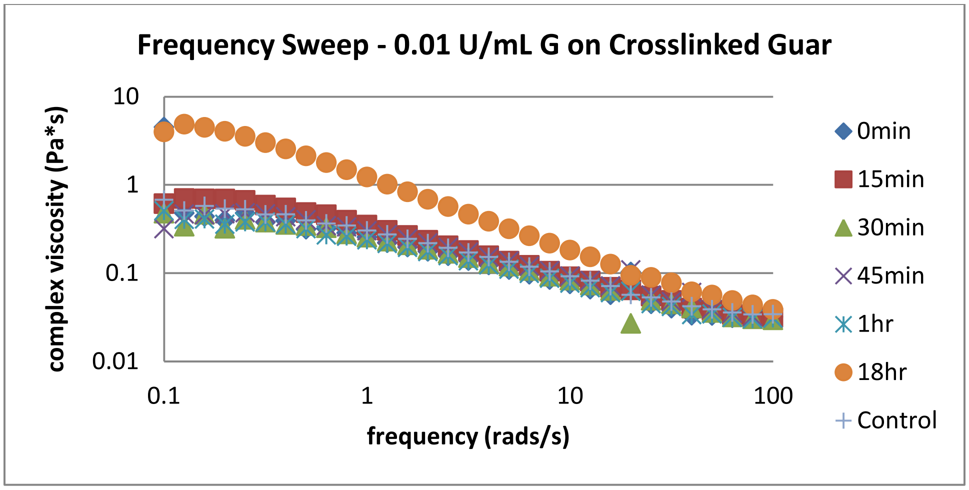

Figure B 21: Frequency sweep for 0.01 U/mL galactosidase on crosslinked guar.

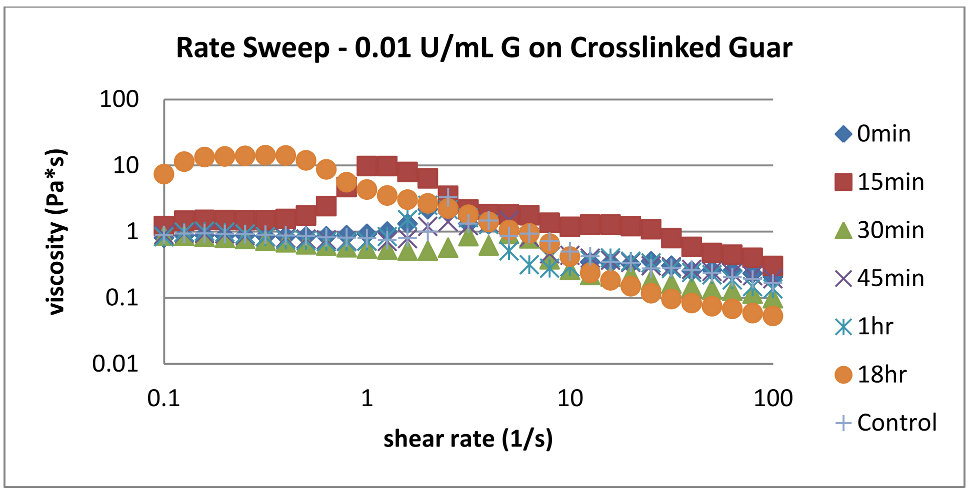

Figure B 22: Rate sweep for 0.01 U/mL galactosidase on crosslinked guar.

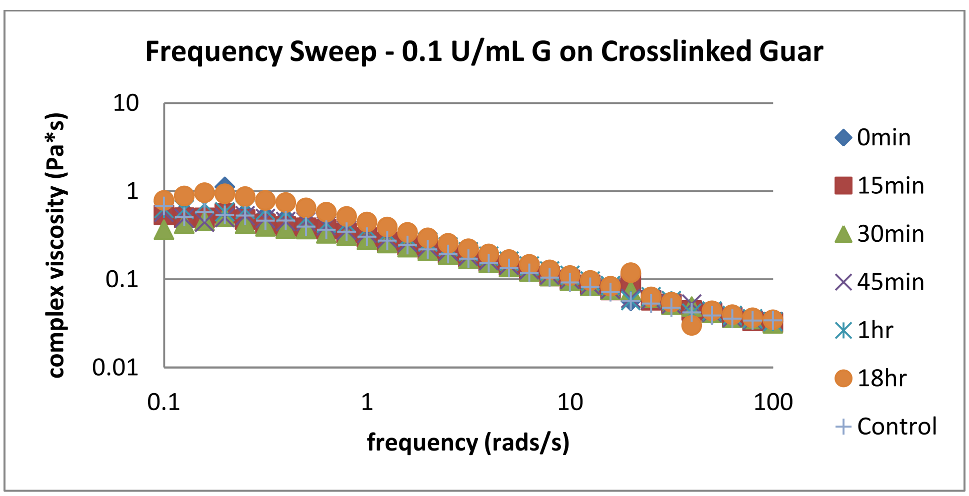

Figure B 23: Frequency sweep for 0.1 U/mL galactosidase on crosslinked guar.

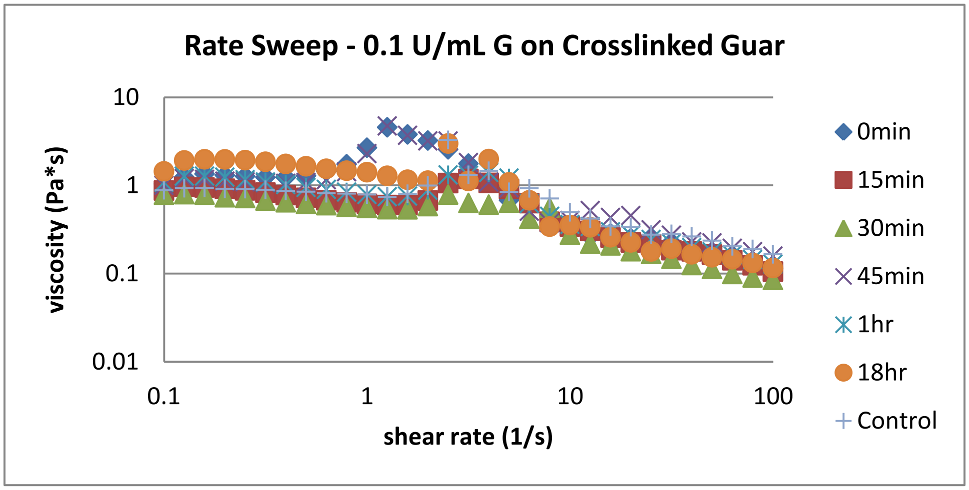

Figure B 24: Rate sweep for 0.01 U/mL galactosidase on crosslinked guar.

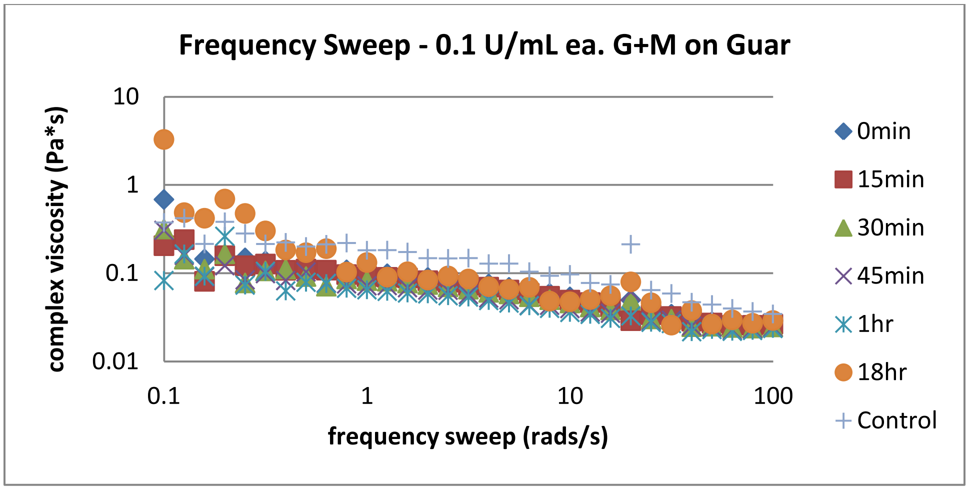

Figure B 25: Frequency sweep for 0.1 U/mL galactosidase + mannanase on guar.

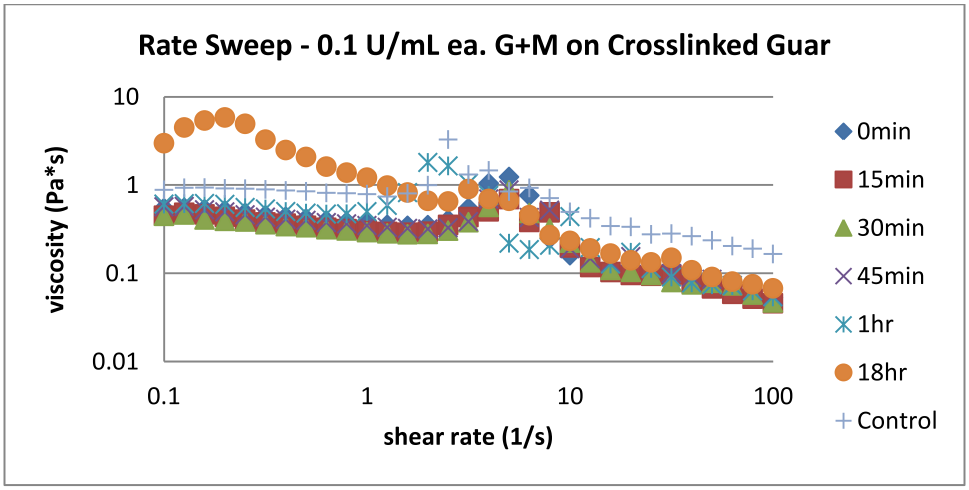

Figure B 26: Rate sweep for 0.1 U/mL galactosidase + mannanase on guar.

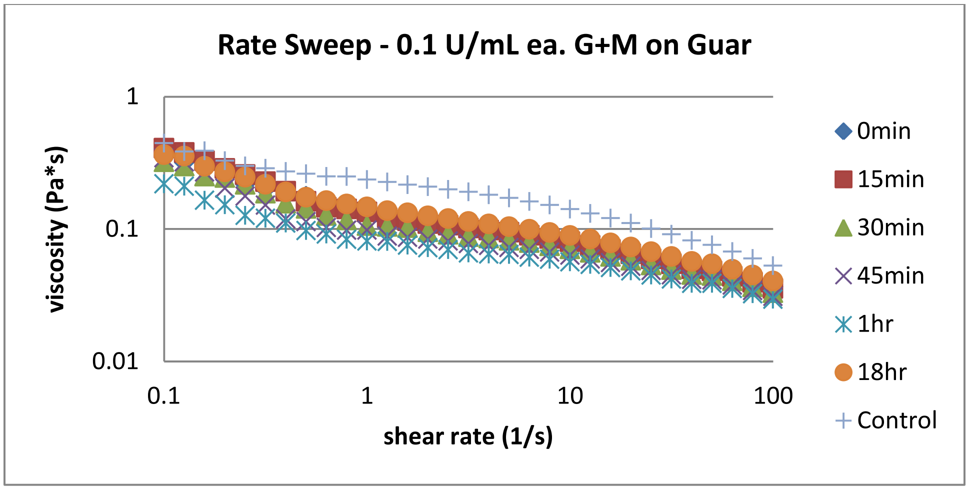

Figure B 27: Frequency sweep for 0.1 U/mL galactosidase + mannanase on crosslinked guar.

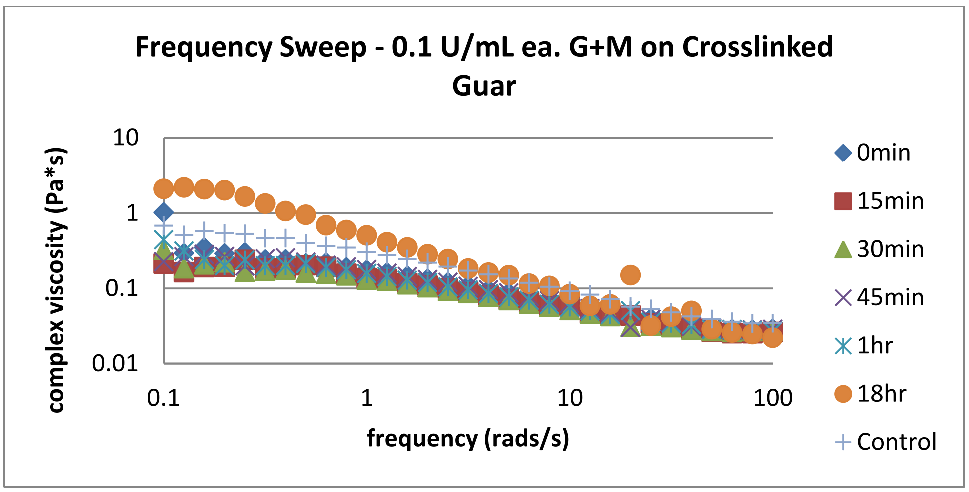

Figure B 28: Rate sweep for 0.1 U/mL galactosidase + mannanase on crosslinked guar.

Appendix C: Rheological & Filtration Test Data Tables

Time (min.)	Fluid	Breaker	Concentration	Average complex viscosity (Pa*s)	Average shear viscosity (Pa*s)
0	CL Guar	APS	Low	0.0698	0.0698
0	CL Guar	APS	Low	0.1124	0.1006
15	CL Guar	APS	Low	0.0528	0.1453
15	CL Guar	APS	Low	0.0519	0.1155
30	CL Guar	APS	Low	0.0507	0.1265
30	CL Guar	APS	Low	0.0545	0.1322
45	CL Guar	APS	Low	0.0263	0.0430
45	CL Guar	APS	Low	0.0338	0.0688
60	CL Guar	APS	Low	0.0292	0.0490
60	CL Guar	APS	Low	0.0262	0.0525
1080	CL Guar	APS	Low	0.0018	0.0014
1080	CL Guar	APS	Low	0.0045	0.0033
0	CL Guar	APS	High	0.0889	0.4105
0	CL Guar	APS	High	0.0986	0.6078
15	CL Guar	APS	High	0.0132	0.0247
15	CL Guar	APS	High	0.0219	0.0350
30	CL Guar	APS	High	0.0048	0.0019
30	CL Guar	APS	High	0.0095	0.0073
45	CL Guar	APS	High	0.0040	0.0010
45	CL Guar	APS	High	0.0044	0.0021
60	CL Guar	APS	High	0.0049	0.0008
60	CL Guar	APS	High	0.0121	0.0073
1080	CL Guar	APS	High	0.0053	0.0034
1080	CL Guar	APS	High	0.0042	0.0015
0	Guar	APS	Low	0.1207	0.1384
0	Guar	APS	Low	0.1525	0.1947
15	Guar	APS	Low	0.0910	0.0689
15	Guar	APS	Low	0.0820	0.0934
30	Guar	APS	Low	0.1337	0.0936
30	Guar	APS	Low	0.0363	0.0170
45	Guar	APS	Low	0.0661	0.0439
45	Guar	APS	Low	0.0094	0.0083
60	Guar	APS	Low	0.0455	0.0504
60	Guar	APS	Low	0.0082	0.0057
1080	Guar	APS	Low	0.0295	0.0032
1080	Guar	APS	Low	0.0036	0.0113

0	Guar	APS	High	0.0462	0.0653
0	Guar	APS	High	0.5187	0.4040
15	Guar	APS	High	0.0173	0.0208
15	Guar	APS	High	0.0509	0.0330
30	Guar	APS	High	0.0063	0.0653
30	Guar	APS	High	0.0333	0.0044
45	Guar	APS	High	0.0063	0.0033
45	Guar	APS	High	0.0224	0.0072
60	Guar	APS	High	0.0034	0.0018
60	Guar	APS	High	0.0236	0.0105
1080	Guar	APS	High	0.0030	0.0094
1080	Guar	APS	High	0.0300	0.0053
0	Guar	G+M	High	0.0568	0.0748
0	Guar	G+M	High	0.0599	0.0927
15	Guar	G+M	High	0.0507	0.0722
15	Guar	G+M	High	0.0559	0.0880
30	Guar	G+M	High	0.0463	0.0613
30	Guar	G+M	High	0.0521	0.0788
45	Guar	G+M	High	0.0427	0.0572
45	Guar	G+M	High	0.0467	0.0679
60	Guar	G+M	High	0.0349	0.0473
60	Guar	G+M	High	0.0445	0.0640
1080	Guar	G+M	High	0.0500	0.0598
1080	Guar	G+M	High	0.0737	0.1201
0	CL Guar	G+M	High	0.0767	0.3205
0	CL Guar	G+M	High	0.0773	0.3239
15	CL Guar	G+M	High	0.0614	0.2750
15	CL Guar	G+M	High	0.0667	0.2071
30	CL Guar	G+M	High	0.0709	0.2632
30	CL Guar	G+M	High	0.0514	0.2274
45	CL Guar	G+M	High	0.0939	0.3614
45	CL Guar	G+M	High	0.0565	0.1897
60	CL Guar	G+M	High	0.0931	0.7240
60	CL Guar	G+M	High	0.0454	0.1614
1080	CL Guar	G+M	High	0.1014	0.4114
1080	CL Guar	G+M	High	0.1924	0.4264
0	CL Guar	G	Low	0.1116	1.1459
0	CL Guar	G	Low	0.0898	0.3635
15	CL Guar	G	Low	0.1583	4.8279
15	CL Guar	G	Low	0.0948	0.4463
30	CL Guar	G	Low	0.1060	0.4157

30	CL Guar	G	Low	0.1001	0.3594
45	CL Guar	G	Low	0.1285	0.7995
45	CL Guar	G	Low	0.1184	0.4818
60	CL Guar	G	Low	0.0952	0.2236
60	CL Guar	G	Low	0.1015	1.1748
1080	CL Guar	G	Low	0.0937	0.2712
1080	CL Guar	G	Low	0.5712	1.9361
0	CL Guar	G	High	0.1058	0.3942
0	CL Guar	G	High	0.1473	1.8694
15	CL Guar	G	High	0.1176	0.3186
15	CL Guar	G	High	0.1297	0.6346
30	CL Guar	G	High	0.1434	0.4350
30	CL Guar	G	High	0.0958	0.2882
45	CL Guar	G	High	0.1181	0.5306
45	CL Guar	G	High	0.1384	1.8809
60	CL Guar	G	High	0.1251	0.4565
60	CL Guar	G	High	0.1595	0.6934
1080	CL Guar	G	High	0.1333	0.9321
1080	CL Guar	G	High	0.1819	0.5450
0	Guar	G	Low	0.0572	0.0814
0	Guar	G	Low	0.0914	0.1389
15	Guar	G	Low	0.0676	0.0985
15	Guar	G	Low	0.0710	0.1172
30	Guar	G	Low	0.0674	0.0973
30	Guar	G	Low	0.0572	0.0898
45	Guar	G	Low	0.0645	0.0890
45	Guar	G	Low	0.1124	0.1564
60	Guar	G	Low	0.0544	0.0828
60	Guar	G	Low	0.0799	0.1310
1080	Guar	G	Low	0.0716	0.1132
1080	Guar	G	Low	0.0834	0.1269
0	Guar	G	High	0.0759	0.1076
0	Guar	G	High	0.0848	0.0967
15	Guar	G	High	0.0732	0.1089
15	Guar	G	High	0.0766	0.0955
30	Guar	G	High	0.0700	0.1015
30	Guar	G	High	0.0689	0.1171
45	Guar	G	High	0.1679	0.1643
45	Guar	G	High	0.0720	0.1204
60	Guar	G	High	0.0592	0.0925
60	Guar	G	High	0.0599	0.0999

1080	Guar	G	High	0.0657	0.1036
1080	Guar	G	High	0.0846	0.1218
0	CL Guar	M	Low	0.1278	1.1126
0	CL Guar	M	Low	0.0736	0.2678
15	CL Guar	M	Low	0.0959	0.7756
15	CL Guar	M	Low	0.0573	0.2479
30	CL Guar	M	Low	0.0837	0.0589
30	CL Guar	M	Low	0.0589	0.2887
45	CL Guar	M	Low	0.0960	1.1561
45	CL Guar	M	Low	0.1028	0.4768
60	CL Guar	M	Low	0.1063	1.4244
60	CL Guar	M	Low	0.0869	0.4500
1080	CL Guar	M	Low	0.4127	1.6815
1080	CL Guar	M	Low	0.0116	0.0165
0	CL Guar	M	High	0.1284	0.4294
0	CL Guar	M	High	0.0769	0.3768
15	CL Guar	M	High	0.0982	0.8526
15	CL Guar	M	High	0.0782	0.3701
30	CL Guar	M	High	0.1095	1.3222
30	CL Guar	M	High	0.0674	0.2513
45	CL Guar	M	High	0.0821	0.3433
45	CL Guar	M	High	0.0662	0.1803
60	CL Guar	M	High	0.0667	0.1903
60	CL Guar	M	High	0.0778	0.1758
1080	CL Guar	M	High	0.2033	1.1875
1080	CL Guar	M	High	0.0531	0.1508
0	Guar	M	Low	0.0334	0.0796
0	Guar	M	Low	0.1764	0.0733
15	Guar	M	Low	0.0715	0.0973
15	Guar	M	Low	0.1080	0.1210
30	Guar	M	Low	0.0677	0.0915
30	Guar	M	Low	0.0962	0.0834
45	Guar	M	Low	0.0733	0.1127
45	Guar	M	Low	0.0938	0.0979
60	Guar	M	Low	0.0701	0.0701
60	Guar	M	Low	0.1153	0.1179
1080	Guar	M	Low	0.0775	0.0973
1080	Guar	M	Low	0.0679	0.0599
0	Guar	M	High	0.0428	0.0530
0	Guar	M	High	0.0794	0.0948
15	Guar	M	High	0.0505	0.0774

15	Guar	M	High	0.0857	0.0915
30	Guar	M	High	0.0592	0.0831
30	Guar	M	High	0.0568	0.0584
45	Guar	M	High	0.0468	0.0587
45	Guar	M	High	0.0809	0.0781
60	Guar	M	High	0.0547	0.0682
60	Guar	M	High	0.0602	0.0401
1080	Guar	M	High	0.1062	0.0875
1080	Guar	M	High	0.0756	0.1062
Control	Guar	Control	Control	0.1063	0.1505
Control	Guar	Control	Control	0.1165	0.1606
Control	Guar	Control	Control	0.0950	0.1154
Control	CL Guar	Control	Control	0.0905	0.4408
Control	CL Guar	Control	Control	0.1333	0.9321
Control	CL Guar	Control	Control	0.1348	0.7914

Table C 1: Average viscosity results from rheological testing.

Trial	Breaker	Mass filter (g)	Mass foil (g)	Post-treatment total mass (g)	Mass residue (g)	Volume added (mL)	Concentration (g/mL)	Mass added (g)	Mass residue/ Mass original (%)	Correction	Average permeate percent
1	Guar	0.5790	0.4450	1.0454	0.0214	10	0.005	0.05	42.8	42.8	
2		0.5565	0.4492	1.0313	0.0256	10	0.005	0.05	51.2	51.2	53.0
1	APS 0.01	0.5778	0.4630	1.0289	-0.0119	7	0.005	0.035	-34.0	0.0	
2		0.5756	0.4367	1.0044	-0.0079	7	0.005	0.035	-22.6	0.0	100.0
1	APS 0.1	0.5745	0.4465	1.0153	-0.0057	7	0.005	0.035	-16.3	0.0	
2		0.5777	0.4635	1.0236	-0.0176	7	0.005	0.035	-50.3	0.0	100.0
1	Galactosidase 0.01	0.5609	0.4547	1.0391	0.0235	10	0.005	0.05	47.0	47.0	
2		0.5759	0.4602	1.0604	0.0243	10	0.005	0.05	48.6	48.6	52.2
1	Galactosidase 0.1	0.5878	0.4368	1.0280	0.0034	10	0.005	0.05	6.8	6.8	
2		0.5502	0.4551	1.0263	0.0210	10	0.005	0.05	42.0	42.0	75.6
1	Mannanase 0.01	0.5621	0.4488	1.0321	0.0212	10	0.005	0.05	42.4	42.4	
2		0.5686	0.4539	1.0451	0.0226	10	0.005	0.05	45.2	45.2	56.2
1	Mannanase 0.1	0.5824	0.6933	1.3038	0.0281	10	0.005	0.05	56.2	56.2	
2		0.5650	0.4594	1.0459	0.0215	10	0.005	0.05	43.0	43.0	50.4
1	G + M 0.1	0.5770	0.4508	1.0116	-0.0162	10	0.005	0.05	-32.4	0.0	
2		0.5678	0.4479	0.9994	-0.0163	10	0.005	0.05	-32.6	0.0	100.0

Table C 2: Filtration experiment results for guar samples.

Trial	Breaker	Mass filter (g)	Mass foil (g)	Post-treatment total mass (g)	Mass residue (g)	Volume added (mL)	Concentration (g/mL)	Mass added (g)	Mass residue/ Mass original (%)	Correction	Average permeate percent
1	CL Guar	0.5676	0.4537	1.0604	0.0391	10	0.005	0.05	78.2	78.2	
2		0.5870	0.4522	1.0808	0.0416	10	0.005	0.05	83.2	83.2	19.3
1	APS 0.01	0.5984	0.4614	1.0391	-0.0207	7	0.005	0.035	-59.1	0.0	
2		0.5636	0.4551	0.9944	-0.0243	7	0.005	0.035	-69.4	0.0	100.0
1	APS 0.1	0.5855	0.4469	1.0135	-0.0189	7	0.005	0.035	-54.0	0.0	
2		0.5923	0.4654	1.0382	-0.0195	7	0.005	0.035	-55.7	0.0	100.0
1	Galactosidase 0.01	0.5761	0.4579	1.0132	-0.0208	10	0.005	0.05	-41.6	0.0	
2		0.5677	0.4459	0.9961	-0.0175	10	0.005	0.05	-35.0	0.0	100.0
1	Galactosidase 0.1	0.5540	0.4692	1.0189	-0.0043	10	0.005	0.05	-8.6	0.0	
2		0.5773	0.463	1.0207	-0.0196	10	0.005	0.05	-39.2	0.0	100.0
1	Mannanase 0.01	0.5793	0.4543	1.0425	0.0089	10	0.005	0.05	17.8	17.8	
2		0.5685	0.4284	1.0044	0.0075	10	0.005	0.05	15.0	15.0	83.6
1	Mannanase 0.1	0.5660	0.4448	0.9948	-0.0160	10	0.005	0.05	-32.0	0.0	
2		0.5939	0.6933	1.2695	-0.0177	10	0.005	0.05	-35.4	0.0	100.0
1	G + M 0.1	0.5590	0.455	1.0186	0.0046	10	0.005	0.05	9.2	9.2	
2		0.5756	0.456	1.0523	0.0207	10	0.005	0.05	41.4	41.4	74.7

Table C 3: Filtration experiment results for crosslinked guar samples.